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Disclosure

The authors report no conflicts of interest in this work.

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Page 906 of 1861

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PREOMICS EXHIBIT 1002
Page 907 of 1861

Supplementary materials

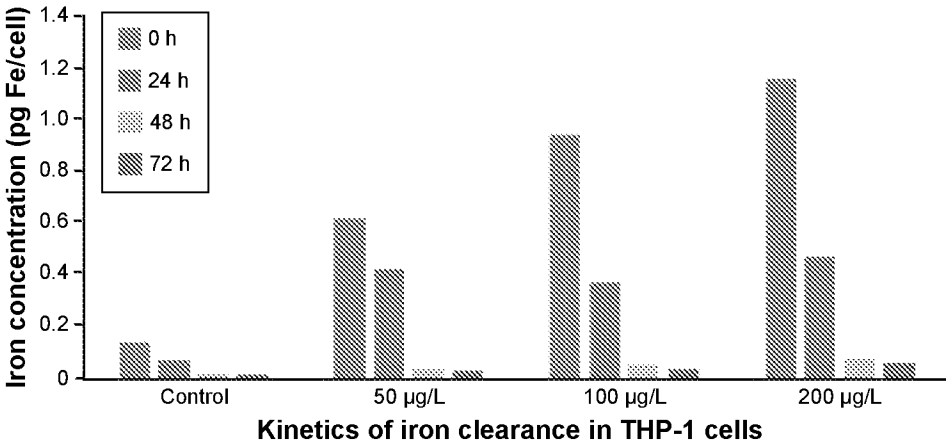


Figure S1 In vitro uptake and clearance of SPIONs in THP-1 monocytic cells.

Notes: THP-1 cells (triplicate samples) were incubated with given concentrations of SPIONs for 24 hours. Afterward, the cells were washed to remove noninternalized SPIONs and cultured for further 72 hours in the absence of nanoparticles. The measurement of iron content per cell was done using atomic emission spectroscopy at the time of SPION removal (0 hour) and after 24, 48, and 72 hours postremoval. Gradual decrease in cellular iron content is observed over 72 hours due to cell metabolism and proliferation.

Abbreviation: SPIONs, superparamagnetic iron oxide nanoparticles.

PREOMICS EXHIBIT 1002
Page 908 of 1861

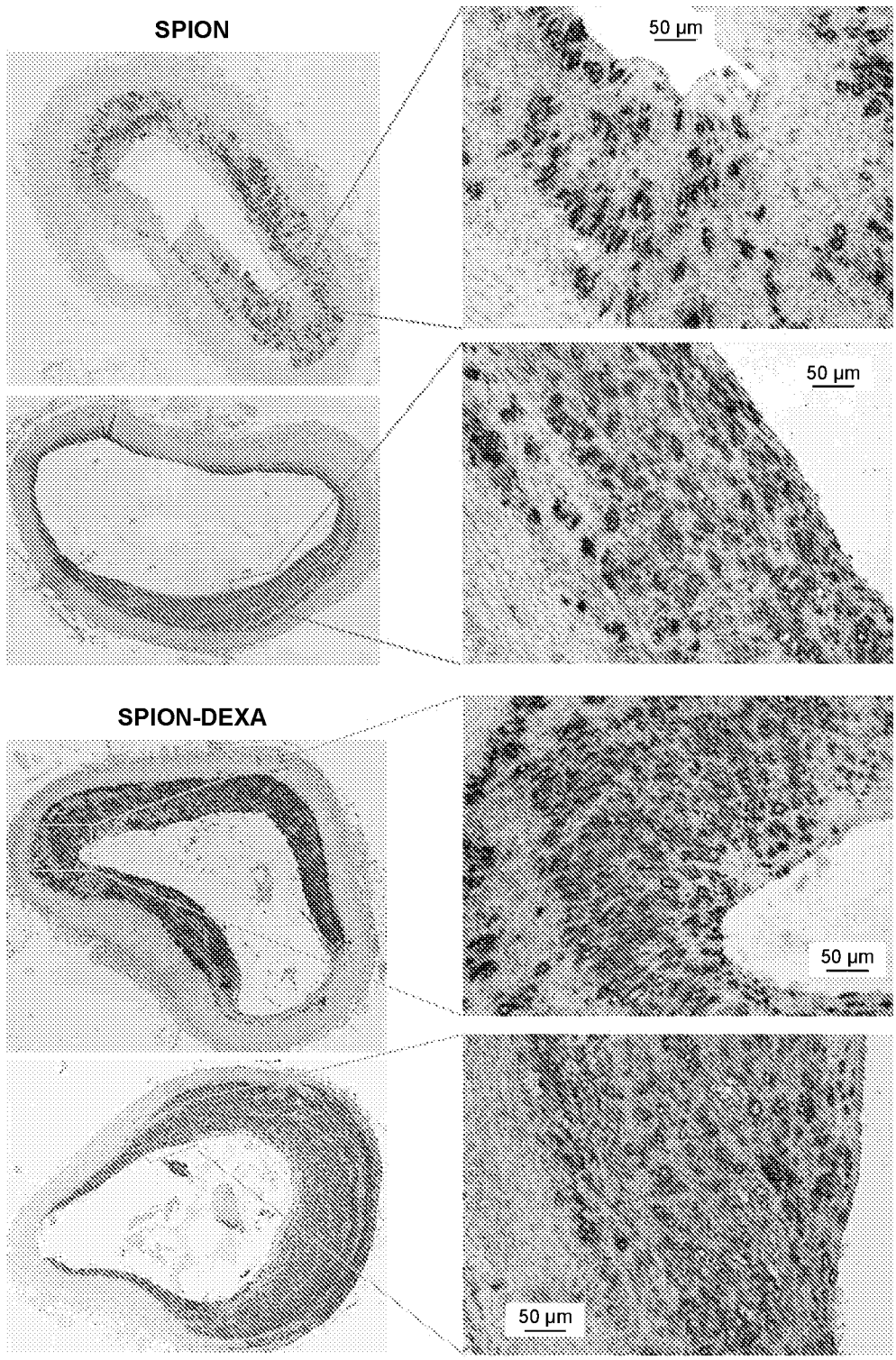


Figure S2 In vivo effects of early MDT with SPION-DEXA on macrophage load.

Notes: Following the balloon injury, the animals were intraarterially administered either control SPION (n=5) or SPION-DEXA (n=4) under external magnetic field. After 5 weeks of high-cholesterol diet and normal diet for 2 weeks, animals were sacrificed and the excised aortas analyzed histochemically. Mouse monoclonal antibody against rabbit macrophages (RAM-11, 1:50 dilution) was used to detect plaque macrophages. Digital images of the plaques at ×10 objective magnification (left, overview images) or 20× objective magnification (right) were obtained using AxioObserver.Z1 microscope. The representative example images from two different animals per each group show macrophage accumulation in the atherosclerotic plaques.

Abbreviations: MDT, magnetic drug targeting; SPION, superparamagnetic iron oxide nanoparticle; SPION-DEXA, SPIONs conjugated with dexamethasone phosphate; THP-1, a human monocytic cell line.

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Page 909 of 1861

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Signature	/Angeline R. Babel/	Date (YYYY-MM-DD)	2021-04-19
Name/Print	Angeline R. Babel	Registration Number	60,886

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	The Brigham and Women's Hospital, Inc.	)
Serial No.:	17/215,923	)
Filed:	March 29, 2021	)
For:	SYSTEM AND SENSOR ARRAY	)
Group Art Unit:	1618	)
Examiner:	TBD	)

Confirmation No. 6706

CERTIFICATE OF EFS FILING

I hereby certify that this correspondence is being electronically transmitted to the United States Patent and Trademark Office, Commissioner for Patents, via the EFS on the date below.

April 23, 2021

/Debra M. Szumowski/
Debra M. Szumowski

RESPONSE TO NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Please consider the following in response to the Notice to File Missing Parts of Nonprovisional Application mailed April 7, 2021.

Remarks begin on page 2 of this communication.

REMARKS

In response to the Notice, payment in the amount of $80 for the Declaration surcharge is being submitted herewith.

Deposit Account Authorization

No additional fees are believed to be due with this communication. However, the Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees, except issue fees, which may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 17-0055.

Respectfully submitted,

QUARLES & BRADY LLP
33 EAST MAIN STREET, SUITE 900
MADISON, WI 53703-3095
(608) 283-2453

Date: April 23, 2021

By: /Angeline R. Babel/
Angeline R. Babel, PhD
Registration No. 60,886

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
17/215,923	03/29/2021	1618	1830	129319.00770.BWH24258	30	1

CONFIRMATION NO. 6706
UPDATED FILING RECEIPT

26710
QUARLES & BRADY LLP
ATTN: IP DOCKET
411 E. WISCONSIN AVENUE
SUITE 2400
MILWAUKEE, WI 53202-4428

OC000000125187457

Date Mailed: 04/28/2021

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Omid Farokhzad, Waban, MA;
Morteza Mahmoudi, Brookline, MA;
Claudia Corbo, Milano, ITALY;

Applicant(s)

THE BRIGHAM AND WOMEN'S HOSPITAL, INC., Boston, MA;

Power of Attorney: The patent practitioners associated with Customer Number 26710

Domestic Applications for which benefit is claimed - None.
A proper domestic benefit claim must be provided in an Application Data Sheet in order to constitute a claim for domestic benefit. See 37 CFR 1.76 and 1.78.

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

PREOMICS EXHIBIT 1002
Page 921 of 1861

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 04/05/2021

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 17/215,923**

Projected Publication Date: 09/29/2022

Non-Publication Request: No

Early Publication Request: No

** **SMALL ENTITY** **

Title

SYSTEM AND SENSOR ARRAY

Preliminary Class

424

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
17/215,923	03/29/2021	Omid Farokhzad	129319.00770.BWH24258

CONFIRMATION NO. 6706

26710
QUARLES & BRADY LLP
ATTN: IP DOCKET
411 E. WISCONSIN AVENUE
SUITE 2400
MILWAUKEE, WI 53202-4428

INFORMAL NOTICE

OC000000125187458

Date Mailed: 04/28/2021

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Omid Farokhzad
  Morteza Mahmoudi
  Claudia Corbo

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/twoldeyes/

To: pat-dept@quarles.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 26710

Apr 28, 2021 03:57:53 AM

Dear PAIR Customer:

QUARLES & BRADY LLP
ATTN: IP DOCKET
411 E. WISCONSIN AVENUE
SUITE 2400
MILWAUKEE, WI 53202-4428
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 26710 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

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Application	Document	Mailroom Date	Attorney Docket No.
17215923	APP.FILE.REC	04/28/2021	129319.00770.BWH24258
	M327	04/28/2021	129319.00770.BWH24258

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/215,923	03/29/2021	Omid Farokhzad	129319.00770.BWH24258	6706

26710 7590 05/11/2021

QUARLES & BRADY LLP
ATTN: IP DOCKET
411 E. WISCONSIN AVENUE
SUITE 2400
MILWAUKEE, WI 53202-4428

EXAMINER	
ART UNIT	PAPER NUMBER
1641	

NOTIFICATION DATE	DELIVERY MODE
05/11/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

pat-dept@quarles.com

Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 17/215,923	**Applicant(s)** Farokhzad et al.	
	Examiner CHERYL P GIBSON BAYLOR	**Art Unit** OPET	**AIA (FITF) Status** Yes

1. THE REQUEST FILED 29 March 2021 IS **GRANTED** .

The above-identified application has met the requirements for prioritized examination

A. ☑ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination**. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;

B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims** , or a multiple dependent claim;

C. filing a **request for continued examination** ;

D. filing a notice of appeal;

E. filing a request for suspension of action;

F. mailing of a notice of allowance;

G. mailing of a final Office action;

H. completion of examination as defined in 37 CFR 41.102; or

I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to CHERYL GIBSON BAYLOR at (571)272-3213. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/CHERYL GIBSON BAYLOR/ Paralegal Specialist, OPET	

To: pat-dept@quarles.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 26710

May 12, 2021 03:55:08 AM

Dear PAIR Customer:

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411 E. WISCONSIN AVENUE
SUITE 2400
MILWAUKEE, WI 53202-4428
UNITED STATES

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The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

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Application	Document	Mailroom Date	Attorney Docket No.
17215923	TRACK1.GRANT	05/11/2021	129319.00770.BWH24258

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Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

PTO/SB/08a (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	17215923
Filing Date	2021-03-29
First Named Inventor	Farokhzad, Omid C.
Art Unit	1642
Examiner Name	Lisa Cook
Attorney Docket Number	129319.00770.BWH24258

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2013/022995	WO		2013-02-14	CARIS LIFE SCIENCES LUXEMBOURG HOLDINGS, S.A.R.I.		

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	17215923
	Filing Date	2021-03-29
	First Named Inventor	Farokhzad, Omid C.
	Art Unit	1642
	Examiner Name	Lisa Cook
	Attorney Docket Number	129319.00770.BWH24258

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If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	17215923
	Filing Date	2021-03-29
	First Named Inventor	Farokhzad, Omid C.
	Art Unit	1642
	Examiner Name	Lisa Cook
	Attorney Docket Number	129319.00770.BWH24258

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	17215923
Filing Date	2021-03-29
First Named Inventor	Farokhzad, Omid C.
Art Unit	1642
Examiner Name	Lisa Cook
Attorney Docket Number	129319.00770.BWH24258

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Angeline R. Babel/	Date (YYYY-MM-DD)	2021-05-17
Name/Print	Angeline R. Babel	Registration Number	60,886

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

BIOMARKER COMPOSITIONS AND METHODS

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application Nos. 61/521,333, filed August 8, 2011; 61/523,763, filed August 15, 2011; 61/526,623, filed August 23, 2011; 61/529,762, filed August 31, 2011; 61/534,352, filed September 13, 2011; 61/537,462, filed September 21, 2011; 61/542,639, filed October 3, 2011; 61/551,674, filed October 26, 2011; 61/559,676, filed November 14, 2011; 61/612,111, filed March 16, 2012; and 61/619,803, filed April 3, 2012; all of which applications are incorporated herein by reference in their entirety.

[0002] This application is a continuation-in-part of International Patent Application PCT/US2012/042519, filed June 14, 2012, which application claims the benefit of U.S. Provisional Patent Application Nos. 61/497,895, filed June 16, 2011; 61/499,138, filed June 20, 2011; 61/501,680, filed June 27, 2011; 61/506,019, filed July 8, 2011; 61/506,606, filed July 11, 2011; 61/506,598, filed July 11, 2011; 61/507,989, filed July 14, 2011; 61/511,455, filed July 25, 2011; 61/523,763, filed August 15, 2011; and 61/526,623, filed August 23, 2011, all of which applications are incorporated herein by reference in their entirety.

[0003] This application is also a continuation-in-part of International Patent Application PCT/US2012/041387, filed June 7, 2012, which application claims the benefit of U.S. Provisional Patent Application Nos. 61/494,196, filed June 7, 2011; 61/494,355, filed June 7, 2011; and 61/507,989, filed July 14, 2011; all of which applications are incorporated herein by reference in their entirety.

[0004] This application is also a continuation-in-part of International Patent Application PCT/US2012/025741, filed February 17, 2012, which application claims the benefit of U.S. Provisional Patent Application Nos. 61/446,313, filed February 24, 2011; 61/501,680, filed June 27, 2011; 61/471,417, filed April 4, 2011; 61/523,763, filed August 15, 2011; and 61/445,273, filed February 22, 2011; all of which applications are incorporated herein by reference in their entirety.

[0005] This application is also a continuation-in-part of International Patent Application PCT/US2011/048327, filed August 18, 2011, which application claims the benefit of U.S. Provisional Patent Application Nos. 61/374,951, filed August 18, 2010; 61/379,670, filed September 2, 2010; 61/381,305, filed September 9, 2010; 61/383,305, filed September 15, 2010; 61/391,504, filed October 8, 2010; 61/393,823, filed October 15, 2010; 61/411,890, filed November 9, 2010; 61/414,870, filed November 17, 2010; 61/416,560, filed November 23, 2010; 61/421,851, filed December 10, 2010; 61/423,557, filed December 15, 2010; 61/428,196, filed December 29, 2010; all of which applications are incorporated herein by reference in their entirety.

[0006] This application is also a continuation-in-part of International Patent Application PCT/US2011/026750, filed March 1, 2011, which application claims is a continuation-in-part application of U.S. Patent Application Serial No. 12/591,226, filed November 12, 2009, which claims the benefit of U.S. Provisional Application Nos. 61/114,045, filed November 12, 2008; 61/114,058, filed November 12, 2008; 61/114,065, filed November 13, 2008; 61/151,183, filed February 9, 2009; 61/278,049, filed October 2, 2009; 61/250,454, filed October 9, 2009; and 61/253,027 filed October 19, 2009; and which application also claims the benefit of U.S. Provisional Application Nos. 61/274,124, filed March 1, 2010; 61/357,517, filed June 22, 2010; 61/364,785, filed July 15, 2010; all of which applications are incorporated herein by reference in their entirety.

PREOMICS EXHIBIT 1002
Page 935 of 1861

[0007] This application is also a continuation-in-part of International Patent Application PCT/US201 1/03 1479, filed April 6, 201 1, which application claims the benefit of U.S. Provisional Patent Application Nos. 61/321,392, filed April 6, 2010; 61/321,407, filed April 6, 2010; 61/332,174, filed May 6, 2010; 61/348,214, filed May 25, 2010, 61/348,685, filed May 26, 2010; 61/354,125, filed June 11, 2010; 61/355,387, filed June 16, 2010; 61/356,974, filed June 21, 2010; 61/357,5 17, filed June 22, 2010; 61/362,674, filed July 8, 2010; 61/413,377, filed November 12, 2010; 61/322,690, filed April 9, 2010; 61/334,547, filed May 13, 2010; 61/364,785, filed July 15, 2010; 61/370,088, filed August 2, 2010; 61/379,670, filed September 2, 2010; 61/381,305, filed September 9, 2010; 61/383,305, filed September 15, 2010; 61/391,504, filed October 8, 2010; 61/393,823, filed October 15, 2010; 61/41 1,890, filed November 9, 2010; and 61/416,560, filed November 23, 2010; all of which applications are incorporated herein by reference in their entirety.

BACKGROUND

[0008] Biomarkers for conditions and diseases such as cancer include biological molecules such as proteins, peptides, lipids, RNAs, DNA and variations and modifications thereof.

[0009] The identification of specific biomarkers, such as DNA, RNA and proteins, can provide biosignatures that are used for the diagnosis, prognosis, or theranosis of conditions or diseases. Biomarkers can be detected in bodily fluids, including circulating DNA, RNA, proteins, and vesicles. Circulating biomarkers include proteins such as PSA and CA125, and nucleic acids such as SEPT9 DNA and PCA3 messenger RNA (mRNA). Circulating biomarkers can be associated with circulating vesicles. Vesicles are membrane encapsulated structures that are shed from cells and have been found in a number of bodily fluids, including blood, plasma, serum, breast milk, ascites, bronchoalveolar lavage fluid and urine. Vesicles can take part in the communication between cells as transport vehicles for proteins, RNAs, DNAs, viruses, and prions. MicroRNAs are short RNAs that regulate the transcription and degradation of messenger RNAs. MicroRNAs have been found in bodily fluids and have been observed as a component within vesicles shed from tumor cells. The analysis of circulating biomarkers associated with diseases, including vesicles and/or microRNA, can aid in detection of disease or severity thereof, determining predisposition to a disease, as well as making treatment decisions.

[0010] Vesicles present in a biological sample provide a source of biomarkers, e.g., the markers are present within a vesicle (vesicle payload), or are present on the surface of a vesicle. Characteristics of vesicles (e.g., size, surface antigens, determination of cell-of-origin, payload) can also provide a diagnostic, prognostic or theranostic readout. There remains a need to identify biomarkers that can be used to detect and treat disease. microRNA, proteins and other biomarkers associated with vesicles as well as the characteristics of a vesicle can provide a diagnosis, prognosis, or theranosis.

[0011] The present invention provides methods and systems for characterizing a phenotype by detecting biomarkers that are indicative of disease or disease progress. The biomarkers can be circulating biomarkers including without limitation vesicle markers, protein, nucleic acids, mRNA, or and microRNA. The biomarkers can be nucleic acid-protein complexes.

SUMMARY

[0012] Disclosed herein are methods and compositions for characterizing a phenotype by analyzing circulating biomarkers, such as a vesicle, microRNA or protein present in a biological sample. Characterizing a phenotype for a subject or individual may include, but is not limited to, the diagnosis of a disease or condition, the

PREOMICS EXHIBIT 1002
Page 936 of 1861

prognosis of a disease or condition, the determination of a disease stage or a condition stage, a drug efficacy, a physiological condition, organ distress or organ rejection, disease or condition progression, therapy-related association to a disease or condition, or a specific physiological or biological state.

[0013] In an aspect, the invention provides a method comprising: (a) contacting a biological sample with one or more reagent, wherein the one or more reagent specifically binds to one or more biomarker in **Table 5**; (b) detecting a presence or level of one or more biomarker in the biological sample based on the contacting of the biological sample and the one or more reagent; and (c) identifying a biosignature comprising the presence or level of the one or more biomarker detected in the biological sample. The method may further comprise comparing the biosignature to a reference biosignature, wherein the comparison is used to characterize a cancer. The reference biosignature can be from a subject without the cancer. The reference biosignature can be from the subject. For example, the reference biosignature can be from a non-malignant sample from the subject such as normal adjacent tissue, or a different sample taken from the subject over a time course. The characterizing may comprise identifying the presence or risk of the cancer in a subject, or identifying the cancer in a subject as metastatic or aggressive. The comparing step may comprise determining whether the biosignature is altered relative to the reference biosignature, thereby providing a prognostic, diagnostic or theranostic determination for the cancer.

[0014] In some embodiments, the one or more biomarker comprises a protein selected from the group consisting of A33, ABL2, ADAM 10, AFP, ALA, ALEX, ALPL, ApoJ/CLU, ASCA, ASPH(A-IO), ASPH(DOIP), AURKB, B7H3, B7H3, B7H4, BCNP, BDNF, CA125(MUC16), CA-19-9, C-Bir, CD10, CD15 1, CD24, CD41, CD44, CD46, CD59(MEM-43), CD63, CD63, CD66eCEA, CD81, CD81, CD9, CD9, CDA, CDADC1, CRMP-2, CRP, CXCL12, CXCR3, CYFRA21-1, DDX-1, DLL4, DLL4, EGFR, Epcam, EphA2, ErbB2, ERG, EZH2, FASL, FLNA, FRT, GAL3, GATA2, GM-CSF, Gro-alpha, HAP, HER3(ErbB3), HSP70, HSPB1, hVEGFR2, iC3b, IL-1B, IL6R, IL6Unc, IL7Ralpha/CD127, IL8, INSIG-2, Integrin, KLK2, LAMN, Mammoglobin, M-CSF, MFG-E8, MIF, MISRII, MMP7, MMP9, MUC1, Mucl, MUC17, MUC2, Ncam, NDUFB7, NGAL, NK-2R(C-21), NT5E (CD73), p53, PBP, PCSA, PCSA, PDGFRB, PIMl, PRL, PSA, PSA, PSMA, PSMA, RAGE, RANK, ReglV, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2(C-15), SPARC, SPC, SPDEF, SPP1, STEAP, STEAP4, TFF3, TGM2, TIMP-1, TMEM21 1, Trail-R2, Trail-R4, TrKB(poly), Trop2, TsglOl, TWEAK, UNC93A, VEGFA, wnt-5a(C-16), and a combination thereof. The one or more biomarker may further comprise a protein selected from the group consisting of CD9, CD63, CD81, PCSA, MUC2, MFG-E8, and a combination thereof. In some embodiments, the biosignature is used to characterize a cancer, e.g., a prostate cancer.

[0015] In other embodiments, the one or more biomarker comprises the one or more microRNA selected from the group consisting of miR-148a, miR-329, miR-9, miR-378*, miR-25, miR-614, miR-5 18c*, miR-378, miR-765, let-7f-2*, miR-574-3p, miR-497, miR-32, miR-379, miR-520g, miR-542-5p, miR-342-3p, miR-1206, miR-663, miR-222, and a combination thereof. The one or more biomarker can also be selected from the group consisting of hsa-miR-877*, hsa-miR-593, hsa-miR-595, hsa-miR-300, hsa-miR-324-5p, hsa-miR-548a-5p, hsa-miR-329, hsa-miR-550, hsa-miR-886-5p, hsa-miR-603, hsa-miR-490-3p, hsa-miR-938, hsa-miR-149, hsa-miR-150, hsa-miR-1296, hsa-miR-384, hsa-miR-487a, hsa-miRPlus-C1089, hsa-miR-485-3p, hsa-miR-525-5p, and a combination thereof. In embodiments, the one or more biomarker is selected from the group consisting of miR-588, miR-1258, miR-16-2*, miR-938, miR-526b, miR-92b*, let-7d, miR-378*, miR-124, miR-376c, miR-26b,

PREOMICS EXHIBIT 1002
Page 937 of 1861

miR-1204, miR-574-3p, miR-195, miR-499-3p, miR-21 10, miR-888, and a combination thereof. The biosignature can be used to characterize a cancer, e.g., a prostate cancer.

[0016] In still other embodiments, the one or more biomarker comprises a protein selected from the group consisting of A33, ADAM 10, AMACR, ASPH (A-10), AURKB, B7H3, CA125, CA-19-9, C-Bir, CD24, CD3, CD41, CD63, CD66e CEA, CD81, CD9, CDADCl, CSA, CXCL12, DCRN, EGFR, EphA2, ERG, FLNA, FRT, GAL3, GM-CSF, Gro-alpha, HER 3 (ErbB3), hVEGFR2, IL6 Unc, Integrin, Mammaglobin, MFG-E8, MMP9, MUC1, MUC17, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, PBP, PCSA, PIM1, PRL, PSA, PSIP1/LEDGF, PSMA, RANK, S100-A4, seprase/FAP, SIM2 (C-15), SPDEF, SSX2, STEAP, TGM2, TIMP-1, Trail-R4, Tsg 101, TWEAK, UNC93A, VCAN, XAGE-1, and a combination thereof. The one or more biomarker may further comprise a protein selected from the group consisting of EpCAM, CD81, PCSA, MUC2, MFG-E8, and a combination thereof. In some embodiments, the biosignature is used to characterize a prostate cancer.

[0017] In some embodiments, the one or more biomarker is selected from the group consisting of let-7d, miR-148a, miR-195, miR-25, miR-26b, miR-329, miR-376c, miR-574-3p, miR-888, miR-9, miR1204, miR-16-2*, miR-497, miR-588, miR-614, miR-765, miR92b*, miR-938, let-7f-2*, miR-300, miR-523, miR-525-5p, miR-1182, miR-1244, miR-520d-3p, miR-379, let-7b, miR-125a-3p, miR-1296, miR-134, miR-149, miR-150, miR-187, miR-32, miR-324-3p, miR-324-5p, miR-342-3p, miR-378, miR-378*, miR-384, miR-45 1, miR-455-3p, miR-485-3p, miR-487a, miR-490-3p, miR-502-5p, miR-548a-5p, miR-550, miR-562, miR-593, miR-593*, miR-595, miR-602, miR-603, miR-654-5p, miR-877*, miR-886-5p, miR-125a-5p, miR-140-3p, miR- 192, miR-196a, miR-21 10, miR-212, miR-222, miR-224*, miR-30b*, miR-499-3p, miR-505*, and a combination thereof. The biosignature can be used to characterize a prostate cancer, such as to distinguish the presence of prostate cancer from other prostate conditions.

[0018] In still other embodiments, the one or more biomarker comprises a protein selected from the group consisting of the one or more biomarker comprises a protein selected from the group consisting of A33, ADAM 10, ALIX, AMACR, ASCA, ASPH (A-10), AURKB, B7H3, BCNP, CA125, CA-19-9, C-Bir (Flagellin), CD24, CD3, CD41, CD63, CD66e CEA, CD81, CD9, CDADCl, CRP, CSA, CXCL12, CYFRA21-1, DCRN, EGFR, EpCAM, EphA2, ERG, FLNA, GAL3, GATA2, GM-CSF, Gro alpha, HER3 (ErbB3), HSP70, hVEGFR2, iC3b, IL-1B, IL6 Unc, IL8, Integrin, KLK2, Mammaglobin, MFG-E8, MMP7, MMP9, MS4A1, MUC1, MUC17, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, p53, PBP, PCSA, PIM1, PRL, PSA, PSMA, RANK, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2 (C-15), SPC, SPDEF, SSX2, SSX4, STEAP, TGM2, TIMP-1, TRAIL R2, Trail-R4, Tsg 101, TWEAK, VCAN, VEGF A, XAGE, and a combination thereof. The one or more biomarker may further comprise a protein selected from the group consisting of EpCAM, CD81, PCSA, MUC2, MFG-E8, and a combination thereof. In some embodiments, the biosignature is used to characterize a cancer, e.g., a prostate cancer.

[0019] The one or more biomarker can be a microRNA selected from the group consisting of hsa-miR-45 1, hsa-miR-223, hsa-miR-593*, hsa-miR-1974, hsa-miR-486-5p, hsa-miR-19b, hsa-miR-320b, hsa-miR-92a, hsa-miR-21, hsa-miR-675*, hsa-miR-16, hsa-miR-876-5p, hsa-miR-144, hsa-miR-126, hsa-miR-137, hsa-miR-1913, hsa-miR-29b-1 *, hsa-miR-15a, hsa-miR-93, hsa-miR-1266, and a combination thereof. The biosignature can be used to characterize a prostate cancer, such as to distinguish a cancer from a non-cancer sample, such as distinguishing prostate cancer from non-prostate disorders.

PREOMICS EXHIBIT 1002
Page 938 of 1861

[0020] In an embodiment, the one or more biomarker comprises one or more protein selected from the group consisting of CD9, CD63, CD81, MMP7, EpCAM, and a combination thereof. The one or more biomarker can be a protein selected from the group consisting of STAT3, EZH2, p53, MACC1, SPDEF, RUNX2, YB-1, AURKA, AURKB, and a combination thereof. The one or more biomarker can be a protein selected from the group consisting of PCSA, Muc2, AdamlO, and a combination thereof. The one or more biomarker can include MMP7. The biosignature can be used to detect a cancer, e.g., a breast or prostate cancer.

[0021] In another embodiment, the one or more biomarker comprises a protein selected from the group consisting of Alkaline Phosphatase (AP), CD63, MyoDl, Neuron Specific Enolase, MAP1B, CNPase, Prohibitin, CD45RO, Heat Shock Protein 27, Collagen II, Laminin Bl/bl, Gail, CDw75, bcl-XL, Laminin-s, Ferritin, CD21, ADP-ribosylation Factor (ARF-6), and a combination thereof. The one or more biomarker may comprise a protein selected from the group consisting of CD56/NCAM-1, Heat Shock Protein 27/hsp27, CD45RO, MAP IB, MyoDl, CD45/T200/LCA, CD3zeta, Laminin-s, bcl-XL, Radl 8, Gail, Thymidylate Synthase, Alkaline Phosphatase (AP), CD63, MMP-16 / MT3-MMP, Cyclin C, Neuron Specific Enolase, SIRP al, Laminin Bl/bl, Amyloid Beta (APP), SODD (Silencer of Death Domain), CDC37, Gab-1, E2F-2, CD6, Mast Cell Chymase, Gamma Glutamylcysteine Synthetase (GCS), and a combination thereof. For example, the one or more biomarker may comprise a protein selected from the group consisting of Alkaline Phosphatase (AP), CD56 (NCAM), CD-3 zeta, Maplb, 14.3.3 pan, filamin, thrombospondin, and a combination thereof. The biosignature can be used to characterize a cancer. For example, the biosignature may be used to distinguish between a prostate cancer and other prostate disorders. The biosignature may also be used to distinguish between a prostate cancer and other cancers, e.g., lung, colorectal, breast and brain cancer.

[0022] The one or more biomarker can include Ago2. The one or more biomarker may further comprise one or more microRNA. In an embodiment, the one or more microRNA can be one or more microRNA in **Table 5.** For example, the one or more microRNA can be selected from the group consisting of miR-22, miR-16, miR-148a, miR-92a, miR-45 1, let7a, and a combination thereof. The microRNA may be in complex with Ago2. The biosignature can be used to characterize a prostate cancer, such as to distinguish a prostate cancer from a non-cancer sample.

[0023] In another embodiment, the one or more biomarker comprises a protein selected from the group consisting of ADAM- 10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, SSX4, and a combination thereof. For example, the one or more biomarker may comprise a protein selected from the group consisting of EGFR, EpCAM, KLK2, PBP, SPDEF, SSX2, SSX4, and a combination thereof. The one or more biomarker may also comprise a protein selected from the group consisting of EpCAM, KLK2, PBP, SPDEF, SSX2, SSX4, and a combination thereof.

[0024] In another aspect, the invention provides a method comprising: (a) contacting a biological sample with one or more reagent, wherein the biological sample comprises one or more microvesicle, and further wherein the one or more reagent comprises a first reagent and a second reagent that specifically bind to one or more biomarker in **Table 5;** (b) detecting a presence or level of the one or more microvesicle based on the contacting of the biological sample with the first and second reagents; and (c) identifying a biosignature comprising the presence or level of the one or more microvesicle detected in the biological sample. The method may further comprise comparing the biosignature to a reference biosignature, wherein the comparison is used to characterize a cancer. The reference biosignature can be from a subject without the cancer. The reference biosignature can be

PREOMICS EXHIBIT 1002
Page 939 of 1861

from the subject. For example, the reference biosignature can be from a non-malignant sample from the subject such as normal adjacent tissue, or a different sample taken from the subject over a time course. The characterizing may comprise identifying the presence or risk of the cancer in a subject, or identifying the cancer in a subject as metastatic or aggressive. The comparing step may comprise determining whether the biosignature is altered relative to the reference biosignature, thereby providing a prognostic, diagnostic or theranostic determination for the cancer.

[0025] In an embodiment, the first reagent comprises a capture agent and the second reagent comprises a detector agent. The first and second reagents may comprise antibodies, aptamers, or a combination thereof. In an embodiment, the capture agent is tethered to a substrate, e.g., a well of a microtiter plate, a planar array, a microbead, a column packing material, or the like. The detector agent may be labeled to facilitate its detection. The label may be a fluorescent label, radiolabel, enzymatic label, or the like. The detector agent may be labeled directly or indirectly. Techniques for capture and detection are further described herein.

[0026] The capture and detector agents can be selected from one or more pair of capture and detector agents in any of **Tables 38, 40-44, 50, 51, 55-67** and **72-74.** The invention also contemplates use of multiple pairs of capture and detector agents. In an embodiment, the one or more pair of capture and detector agents comprises binding agent pairs to Mammaglobin - MFG-E8, SIM2 - MFG-E8 and NK-2R - MFG-E8. In another embodiment, the one or more pair of capture and detector agents comprises binding agent pairs to Integrin - MFG-E8, NK-2R - MFG-E8 and Gal3 - MFG-E8. In still another embodiment, the one or more pair of capture and detector agents comprises capture agents to AURKB, A33, CD63, Gro-alpha, and Integrin; and detector agents to MUC2, PCSA, and CD81 . The one or more pair of capture and detector agents may also comprise capture agents to AURKB, CD63, FLNA, A33, Gro-alpha, Integrin, CD24, SSX2, and SIM2; and detector agents to MUC2, PCSA, CD81, MFG-E8, and EpCam. The one or more pair of capture and detector agents can comprise binding agent pairs to EpCam - MMP7, PCSA - MMP7, and EpCam - BCNP. In an embodiment, the one or more pair of capture and detector agents comprises binding agent pairs to EpCam - MMP7, PCSA - MMP7, EpCam - BCNP, PCSA - ADAMI 0, and PCSA - KLK2. In another embodiment, the one or more pair of capture and detector agents comprises binding agent pairs to EpCam - MMP7, PCSA - MMP7, EpCam - BCNP, PCSA - ADAM 10, PCSA - KLK2, PCSA - SPDEF, CD81 - MMP7, PCSA - EpCam, MFGE8 - MMP7 and PCSA - IL-8. In still another embodiment, the one or more pair of capture and detector agents comprises binding agent pairs to EpCam - MMP7, PCSA - MMP7, EpCam - BCNP, PCSA - ADAM 10, and CD81 - MMP7. Unless otherwise specified, the binding agent pairs disclosed herein may comprise both "target of capture agent" - "target of detector agent" and "target of detector agent" - "target of capture agent."

[0027] In one embodiment, the one or more pair of capture and detector agents comprises capture agents to one or more of ADAM-10, BCNP, CD9, EGFR, EpCam, ILIB, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, and SSX4. The pairs may further comprise a detector agent to EpCam. The pairs may also comprise a detector agent to PCSA. The biosignature can be used to characterize a prostate cancer, such as to detect microvesicles shed from prostate cancer cells, to distinguish a prostate cancer from a non-cancer sample, to stage or grade the cancer, or to provide a diagnosis, prognosis or theranosis.

[0028] In another embodiment, the one or more pair of capture and detector agents comprises binding agent pairs selected from the group consisting of EpCAM - EpCAM, EpCAM - KLK2, EpCAM - PBP, EpCAM - SPDEF, EpCAM - SSX2, EpCAM - SSX4, EpCAM - ADAM-10 , EpCAM - SERPINB3, EpCAM - PCSA,

PREOMICS EXHIBIT 1002
Page 940 of 1861

EpCAM - p53, EpCAM - MMP7, EpCAM - IL1B, EpCAM - EGFR, EpCAM - CD9, EpCAM - BCNP, KLK2 - EpCAM, KLK2 - KLK2, KLK2 - PBP, KLK2 - SPDEF, KLK2 - SSX2, KLK2 - SSX4, KLK2 - ADAM-10 , KLK2 - SERPINB3, KLK2 - PCSA, KLK2 - p53, KLK2 - MMP7, KLK2 - IL1B, KLK2 - EGFR, KLK2 - CD9, KLK2 - BCNP, PBP - EpCAM, PBP - KLK2, PBP - PBP, PBP - SPDEF, PBP - SSX2, PBP - SSX4, PBP - ADAM-10 , PBP - SERPINB3, PBP - PCSA, PBP - p53, PBP - MMP7, PBP - IL1B, PBP - EGFR, PBP - CD9, PBP - BCNP, SPDEF - EpCAM, SPDEF - KLK2, SPDEF - PBP, SPDEF - SPDEF, SPDEF - SSX2, SPDEF - SSX4, SPDEF - ADAM-10 , SPDEF - SERPINB3, SPDEF - PCSA, SPDEF - p53, SPDEF - MMP7, SPDEF - IL1B, SPDEF - EGFR, SPDEF - CD9, SPDEF - BCNP, SSX2 - EpCAM, SSX2 - KLK2, SSX2 - PBP, SSX2 - SPDEF, SSX2 - SSX2, SSX2 - SSX4, SSX2 - ADAM-10 , SSX2 - SERPINB3, SSX2 - PCSA, SSX2 - p53, SSX2 - MMP7, SSX2 - IL1B, SSX2 - EGFR, SSX2 - CD9, SSX2 - BCNP, SSX4 - EpCAM, SSX4 - KLK2, SSX4 - PBP, SSX4 - SPDEF, SSX4 - SSX2, SSX4 - SSX4, SSX4 - ADAM-10 , SSX4 - SERPINB3, SSX4 - PCSA, SSX4 - p53, SSX4 - MMP7, SSX4 - IL1B, SSX4 - EGFR, SSX4 - CD9, SSX4 - BCNP, ADAM-10 - EpCAM, ADAM-10 - KLK2, ADAM-10 - PBP, ADAM-10 - SPDEF, ADAM-10 - SSX2, ADAM-10 - SSX4, ADAM-10 - ADAM-10 , ADAM-10 - SERPINB3, ADAM-10 - PCSA, ADAM-10 - p53, ADAM-10 - MMP7, ADAM-10 - IL1B, ADAM-10 - EGFR, ADAM-10 - CD9, ADAM-10 - BCNP, SERPINB3 - EpCAM, SERPINB3 - KLK2, SERPINB3 - PBP, SERPINB3 - SPDEF, SERPINB3 - SSX2, SERPINB3 - SSX4, SERPINB3 - ADAM-10 , SERPINB3 - SERPINB3, SERPINB3 - PCSA, SERPINB3 - p53, SERPINB3 - MMP7, SERPINB3 - IL1B, SERPINB3 - EGFR, SERPINB3 - CD9, SERPINB3 - BCNP, PCSA - EpCAM, PCSA - KLK2, PCSA - PBP, PCSA - SPDEF, PCSA - SSX2, PCSA - SSX4, PCSA - ADAM-10 , PCSA - SERPINB3, PCSA - PCSA, PCSA - p53, PCSA - MMP7, PCSA - IL1B, PCSA - EGFR, PCSA - CD9, PCSA - BCNP, p53 - EpCAM, p53 - KLK2, p53 - PBP, p53 - SPDEF, p53 - SSX2, p53 - SSX4, p53 - ADAM-10 , p53 - SERPINB3, p53 - PCSA, p53 - p53, p53 - MMP7, p53 - IL1B, p53 - EGFR, p53 - CD9, p53 - BCNP, MMP7 - EpCAM, MMP7 - KLK2, MMP7 - PBP, MMP7 - SPDEF, MMP7 - SSX2, MMP7 - SSX4, MMP7 - ADAM-10 , MMP7 - SERPINB3, MMP7 - PCSA, MMP7 - p53, MMP7 - MMP7, MMP7 - IL1B, MMP7 - EGFR, MMP7 - CD9, MMP7 - BCNP, IL1B - EpCAM, IL1B - KLK2, IL1B - PBP, IL1B - SPDEF, IL1B - SSX2, IL1B - SSX4, IL1B - ADAM-10 , IL1B - SERPINB3, IL1B - PCSA, IL1B - p53, IL1B - MMP7, IL1B - IL1B, IL1B - EGFR, IL1B - CD9, IL1B - BCNP, EGFR - EpCAM, EGFR - KLK2, EGFR - PBP, EGFR - SPDEF, EGFR - SSX2, EGFR - SSX4, EGFR - ADAM-10 , EGFR - SERPINB3, EGFR - PCSA, EGFR - p53, EGFR - MMP7, EGFR - IL1B, EGFR - EGFR, EGFR - CD9, EGFR - BCNP, CD9 - EpCAM, CD9 - KLK2, CD9 - PBP, CD9 - SPDEF, CD9 - SSX2, CD9 - SSX4, CD9 - ADAM-10 , CD9 - SERPINB3, CD9 - PCSA, CD9 - p53, CD9 - MMP7, CD9 - IL1B, CD9 - EGFR, CD9 - CD9, CD9 - BCNP, BCNP - EpCAM, BCNP - KLK2, BCNP - PBP, BCNP - SPDEF, BCNP - SSX2, BCNP - SSX4, BCNP - ADAM-10 , BCNP - SERPINB3, BCNP - PCSA, BCNP - p53, BCNP - MMP7, BCNP - IL1B, BCNP - EGFR, BCNP - CD9, BCNP - BCNP, and a combination thereof. As listed in this paragraph, the pairs comprise "target of capture agent" - "target of detector agent." The biosignature can be used to characterize a prostate cancer.

[0029] In an embodiment, the one or more pair of capture and detector agents comprises capture agents to one or more of EpCAM, KLK2, PBP, SPDEF, SSX2, SSX4, EGFR; and a detector agent to EpCam. The biosignature can be used to characterize a prostate cancer.

PREOMICS EXHIBIT 1002
Page 941 of 1861

[0030] As noted, the one or more microvesicle may be detected using multiple pairs of capture and detector agents. In an embodiment, the one or more pair of capture and detector agents comprises a plurality of capture agents selected from the group consisting of SSX4 and EpCAM; SSX4 and KLK2; SSX4 and PBP; SSX4 and SPDEF; SSX4 and SSX2; SSX4 and EGFR; SSX4 and MMP7; SSX4 and BCNP1 ; SSX4 and SERPINB3; KLK2 and EpCAM; KLK2 and PBP; KLK2 and SPDEF; KLK2 and SSX2; KLK2 and EGFR; KLK2 and MMP7; KLK2 and BCNP1 ; KLK2 and SERPINB3; PBP and EGFR; PBP and EpCAM; PBP and SPDEF; PBP and SSX2; PBP and SERPINB3; PBP and MMP7; PBP and BCNP1 ; EpCAM and SPDEF; EpCAM and SSX2; EpCAM and SERPINB3; EpCAM and EGFR; EpCAM and MMP7; EpCAM and BCNP1 ; SPDEF and SSX2; SPDEF and SERPINB3; SPDEF and EGFR; SPDEF and MMP7; SPDEF and BCNP1 ; SSX2 and EGFR; SSX2 and MMP7; SSX2 and BCNP1 ; SSX2 and SERPINB3; SERPINB3 and EGFR; SERPINB3 and MMP7; SERPINB3 and BCNP1 ; EGFR and MMP7; EGFR and BCNP1 ; MMP7 and BCNP1 ; and a combination thereof. In a preferred embodiment, the detector agent comprises an EpCAM detector. In some embodiments, the detector agent recognizes one or more of a tetraspanin, CD9, CD63, CD81, CD63, CD9, CD81, CD82, CD37, CD53, Rab-5b, Annexin V, MFG-E8, or a protein in **Table 3.** In another embodiment, the detector agent recognizes one or more of CD9, CD63, CD81, PSMA, PCSA, B7H3, EpCam, ADAM-10, BCNP, EGFR, ILIB, KLK2, MMP7, p53, PBP, SERPINB3, SPDEF, SSX2, and SSX4. When using multiple capture agents, the assay can be multiplexed with a single detector agent. Alternately, each capture agent can be paired with a different detector agent. The biosignature can be used to characterize a prostate cancer.

[0031] In an embodiment, the one or more pair of capture and detector agents comprises binding agent pairs selected from the group consisting of EpCam - EpCam, EpCam - KLK2, EpCam - PBP, EpCam - SPDEF, EpCam - SSX2, EpCam - SSX4, EpCam - EGFR, and a combination thereof. The EpCAM may be the target of the detector agent. The biosignature can be used to characterize a prostate cancer.

[0032] In an embodiment, the one or more pair of capture and detector agents comprises binding agents to EpCam - EpCam.

[0033] In an embodiment, the one or more pair of capture and detector agents comprises binding agents to EpCam - KLK2.

[0034] In an embodiment, the one or more pair of capture and detector agents comprises binding agents to EpCam - PBP.

[0035] In an embodiment, the one or more pair of capture and detector agents comprises binding agents to EpCam - SPDEF.

[0036] In an embodiment, the one or more pair of capture and detector agents comprises binding agents to EpCam - SSX2.

[0037] In an embodiment, the one or more pair of capture and detector agents comprises binding agents to EpCam - SSX4.

[0038] In an embodiment, the one or more pair of capture and detector agents comprises binding agents to EpCam - EGFR.

[0039] In embodiments of the methods of the invention, the biological sample comprises a bodily fluid. Appropriate bodily fluids include without limitation peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, female ejaculate,

PREOMICS EXHIBIT 1002
Page 942 of 1861

sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, umbilical cord blood, or a derivative of any thereof. For example, the biological sample may comprise urine, blood or a blood derivative (e.g., serum or plasma), or a derivative of any thereof.

[0040] In some embodiments of the methods of the invention, the biological sample comprises a tissue sample, cells from a tissue sample, one or more circulating biomarkers released from such cells, or a derivative of any thereof. For example, the methods of the invention can be performed to identify a biosignature for a tissue sample. The biological sample may comprise a cell culture sample, e.g., the sample may comprise cultured cells and/or culture medium comprising circulating biomarkers released from such cultured cells. The tissue sample or culture sample may be a cancer sample may or comprise a tumor sample or tumor cells.

[0041] In the methods of the invention, the biological sample may comprise one or more microvesicle. The biological sample may also consist of the one or more microvesicle. In some embodiments, the one or more biomarker is associated with the one or more microvesicle. The one or more microvesicle may have a diameter between 10 nm and 2000 nm, e.g., between 20 nm and 1500 nm, between 20 nm and 1000 nm, between 20 nm and 500 nm, or between 20 nm and 200 nm.

[0042] The one or more microvesicle can be isolated from the sample using methods disclosed herein or known in the art. In embodiments, the one or more microvesicle is subjected to size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, affinity capture, affinity selection, immunoassay, ELISA, microfluidic separation, flow cytometry or combinations thereof.

[0043] The one or more microvesicle may be contacted with the one or more reagent. In some embodiments, the one or more reagent comprises a nucleic acid, DNA molecule, RNA molecule, antibody, antibody fragment, aptamer, peptoid, zDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), lectin, peptide, dendrimer, membrane protein labeling agent, chemical compound, or a combination thereof. For example, the binding agent can be an antibody or an aptamer. The one or more binding agent can be used to capture and/or detect the one or more microvesicle. In an embodiment, the one or more binding agent binds to one or more surface antigen on the one or more microvesicle. The one or more surface antigen can comprise one or more protein.

[0044] The one or more protein can be any useful biomarker on the vesicles of interest, such as those disclosed herein. In an embodiment, the one or more protein comprises one or more cell specific or cancer specific vesicle marker, e,g., CD9, CD63, CD81, PSMA, PCSA, B7H3, EpCam, or a protein in **Tables 4** or **5.** The one or more protein may also comprise a general vesicle marker, e.g., one or more of a tetraspanin, CD9, CD63, CD81, CD63, CD9, CD81, CD82, CD37, CD53, Rab-5b, Annexin V, MFG-E8, or a protein in **Table 3.** In embodiments, the one or more protein comprises one or more protein in any of **Tables 3-5.** For example, the one or more protein may comprise one or more of CD9, CD63, CD81, PSMA, PCSA, B7H3, EpCam, ADAM-10, BCNP, EGFR, IL1B, KLK2, MMP7, p53, PBP, SERPINB3, SPDEF, SSX2, and SSX4.

[0045] The one or more reagent can be used to capture the one or more microvesicle. The captured microvesicles can be used for further assessment. For example, the payload within the microvesicles can be assessed. Microvesicle payload comprises one or more nucleic acid, peptide, protein, lipid, antigen, carbohydrate, and/or proteoglycan. The nucleic acid may comprise one or more DNA, mRNA, microRNA,

PREOMICS EXHIBIT 1002
Page 943 of 1861

snoRNA, snRNA, rRNA, tRNA, siRNA, hnRNA, or shRNA. In an embodiment, the one or more biomarker comprises payload within the one or more captured microvesicle. For example, the one or more biomarker can include mRNA payload. The one or more biomarker can also include microRNA payload. The one or more biomarker can also include protein payload, e.g., inner membrane protein or soluble protein.

[0046] The methods of the invention can be performed in vitro, e.g., using an in vitro biological sample or a cell culture sample.

[0047] In a further embodiment, the cancer under analysis may be a lung cancer including non-small cell lung cancer and small cell lung cancer (including small cell carcinoma (oat cell cancer), mixed small cell/large cell carcinoma, and combined small cell carcinoma), colon cancer, breast cancer, prostate cancer, liver cancer, pancreas cancer, brain cancer, kidney cancer, ovarian cancer, stomach cancer, skin cancer, bone cancer, gastric cancer, breast cancer, pancreatic cancer, glioma, glioblastoma, hepatocellular carcinoma, papillary renal carcinoma, head and neck squamous cell carcinoma, leukemia, lymphoma, myeloma, or a solid tumor.

[0048] In embodiments, the cancer that is characterized by the subject methods comprises an acute lymphoblastic leukemia; acute myeloid leukemia; adrenocortical carcinoma; AIDS-related cancers; AIDS-related lymphoma; anal cancer; appendix cancer; astrocytomas; atypical teratoid/rhabdoid tumor; basal cell carcinoma; bladder cancer; brain stem glioma; brain tumor (including brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, astrocytomas, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma); breast cancer; bronchial tumors; Burkitt lymphoma; cancer of unknown primary site; carcinoid tumor; carcinoma of unknown primary site; central nervous system atypical teratoid/rhabdoid tumor; central nervous system embryonal tumors; cervical cancer; childhood cancers; chordoma; chronic lymphocytic leukemia; chronic myelogenous leukemia; chronic myeloproliferative disorders; colon cancer; colorectal cancer; craniopharyngioma; cutaneous T-cell lymphoma; endocrine pancreas islet cell tumors; endometrial cancer; ependymoblastoma; ependymoma; esophageal cancer; esthesioneuroblastoma; Ewing sarcoma; extracranial germ cell tumor; extragonadal germ cell tumor; extrahepatic bile duct cancer; gallbladder cancer; gastric (stomach) cancer; gastrointestinal carcinoid tumor; gastrointestinal stromal cell tumor; gastrointestinal stromal tumor (GIST); gestational trophoblastic tumor; glioma; hairy cell leukemia; head and neck cancer; heart cancer; Hodgkin lymphoma; hypopharyngeal cancer; intraocular melanoma; islet cell tumors; Kaposi sarcoma; kidney cancer; Langerhans cell histiocytosis; laryngeal cancer; lip cancer; liver cancer; malignant fibrous histiocytoma bone cancer; medulloblastoma; medulloepithelioma; melanoma; Merkel cell carcinoma; Merkel cell skin carcinoma; mesothelioma; metastatic squamous neck cancer with occult primary; mouth cancer; multiple endocrine neoplasia syndromes; multiple myeloma; multiple myeloma/plasma cell neoplasm; mycosis fungoides; myelodysplastic syndromes; myeloproliferative neoplasms; nasal cavity cancer; nasopharyngeal cancer; neuroblastoma; Non-Hodgkin lymphoma; nonmelanoma skin cancer; non-small cell lung cancer; oral cancer; oral cavity cancer; oropharyngeal cancer; osteosarcoma; other brain and spinal cord tumors; ovarian cancer; ovarian epithelial cancer; ovarian germ cell tumor; ovarian low malignant potential tumor; pancreatic cancer; papillomatosis; paranasal sinus cancer; parathyroid cancer; pelvic cancer; penile cancer; pharyngeal cancer; pineal parenchymal tumors of intermediate differentiation; pineoblastoma; pituitary tumor; plasma cell neoplasm/multiple myeloma; pleuropulmonary blastoma; primary central nervous system (CNS) lymphoma; primary hepatocellular liver

PREOMICS EXHIBIT 1002
Page 944 of 1861

cancer; prostate cancer; rectal cancer; renal cancer; renal cell (kidney) cancer; renal cell cancer; respiratory tract cancer; retinoblastoma; rhabdomyosarcoma; salivary gland cancer; Sezary syndrome; small cell lung cancer; small intestine cancer; soft tissue sarcoma; squamous cell carcinoma; squamous neck cancer; stomach (gastric) cancer; supratentorial primitive neuroectodermal tumors; T-cell lymphoma; testicular cancer; throat cancer; thymic carcinoma; thymoma; thyroid cancer; transitional cell cancer; transitional cell cancer of the renal pelvis and ureter; trophoblastic tumor; ureter cancer; urethral cancer; uterine cancer; uterine sarcoma; vaginal cancer; vulvar cancer; Waldenstrom macroglobulinemia; or Wilm's tumor. For example, the cancer can be a prostate cancer, a lung cancer, a brain cancer, a breast cancer, a colorectal cancer or an ovarian cancer. In preferred embodiments, the cancer is a prostate cancer.

[0049] The methods of the invention can be performed in vitro, e.g., using an in vitro biological sample or a cell culture sample.

[0050] In an aspect, the invention provides a reagent to carry out any of the methods of the invention. For example, the invention provides use of a reagent to carry out the methods. In a related aspect, the invention provides a kit comprising a reagent to carry out any of the methods of the invention. The reagent may be the binding reagent, including without limitation an antibody or aptamer to the one or more biomarker. For example, the reagent can be a binding agent that is capable of binding to at least one of the biomarkers in any of **Tables 3-5, 9-11, 16- 27, 29, 31-32, 37-38, 40-47, 49-52, 54-67,** and **69-74.** In some embodiments, the binding agent is labeled directly or is configured to be indirectly labeled.

[0051] In another aspect, the invention provides an isolated PCSA+, Muc2+, Adaml0+ vesicle. In a related aspect, the invention provides a MMP7+ vesicle. The invention further provides an Ago2+ vesicle. The vesicle may contain payload comprising one or more microRNA selected from **Table 5.** For example, the microRNA can be selected from the group consisting of miR-22, let7a, miR-141, miR- 182, miR-663, miR-155, mirR-125a-5p, miR-548a-5p, miR-628-5p, miR-5 17*, miR-450a, miR-920, hsa-miR-619, miR-1913, miR-224*, miR-502-5p, miR-888, miR-376a, miR-542-5p, miR-30b*, miR-1 179, and a combination thereof. The vesicle may also contain payload comprising one or more messenger RNA (mRNA) in **Table 5.** For example, the mRNA can be selected from the group consisting of **Tables 20-24.**

INCORPORATION BY REFERENCE

[0052] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0053] **FIG. 1A** depicts a method of identifying a biosignature comprising nucleic acid to characterize a phenotype. **FIG. 1B** depicts a method of identifying a biosignature of a vesicle or vesicle population to characterize a phenotype.

[0054] **FIGs. 2A-F** illustrate methods of characterizing a phenotype by assessing vesicle biosignatures. **FIG. 2A** is a schematic of a planar substrate coated with a capture antibody, which captures vesicles expressing that protein. The capture antibody is for a vesicle protein that is specific or not specific for vesicles derived from diseased cells ("disease vesicle"). The detection antibody binds to the captured vesicle and provides a fluorescent signal. The detection antibody can detect an antigen that is generally associated with vesicles, or is

PREOMICS EXHIBIT 1002
Page 945 of 1861

associated with a cell-of-origin or a disease, e.g., a cancer. **FIG. 2B** is a schematic of a bead coated with a capture antibody, which captures vesicles expressing that protein. The capture antibody is for a vesicle protein that is specific or not specific for vesicles derived from diseased cells ("disease vesicle"). The detection antibody binds to the captured vesicle and provides a fluorescent signal. The detection antibody can detect an antigen that is generally associated with vesicles, or is associated with a cell-of-origin or a disease, e.g., a cancer. **FIG. 2C** is an example of a screening scheme that can be performed by multiplexing using the beads as shown in **FIG. 2B. FIG. 2D** presents illustrative schemes for capturing and detecting vesicles to characterize a phenotype. **FIG. 2E** presents illustrative schemes for assessing vesicle payload to characterize a phenotype. **FIG. 2F** presents illustrative schemes for capturing and detecting vesicles and optionally assessing payload to characterize a phenotype.

[0055] **FIG. 3** illustrates a computer system that can be used in some exemplary embodiments of the invention.

[0056] **FIG. 4** illustrates a method of depicting results using a bead based method of detecting vesicles from a subject. The number of beads captured at a given intensity is an indication of how frequently a vesicle expresses the detection protein at that intensity. The more intense the signal for a given bead, the greater the expression of the detection protein. The figure shows a normalized graph obtained by combining normal patients into one curve and cancer patients into another, and using bio-statistical analysis to differentiate the curves. Data from each individual is normalized to account for variation in the number of beads read by the detection machine, added together, and then normalized again to account for the different number of samples in each population.

[0057] **FIG. 5** illustrates the capture of prostate cancer cells-derived vesicles from plasma with EpCam by assessing TMPRSS2-ERG expression. VCaP purified vesicles were spiked into normal plasma and then incubated with Dynal magnetic beads coated with either the EpCam or isotype control antibody. RNA was isolated directly from the Dynal beads. Equal volumes of RNA from each sample were used for RT-PCR and subsequent Taqman assays.

[0058] **FIG. 6** depicts a bar graph of miR-21 or miR-141 expression with CD9 bead capture. 1 ml of plasma from prostate cancer patients, 250 ng/ml of LNCaP, or normal purified vesicles were incubated with CD9 coated Dynal beads. The RNA was isolated from the beads and the bead supernatant. One sample (#6) was also uncaptured for comparison. microRNA expression was measured with qRT-PCR and the mean CT values for each sample compared. CD9 capture improves the detection of miR-21 and miR-141 in prostate cancer samples.

[0059] **FIG. 7A** illustrates separation and identification of vesicles using the MoFlo XDP. **FIG. 7B** illustrates FACS analysis of VCaP cells and exosomes stained with antibodies to CD9, B7H3, PCSA and PSMA. **FIG. 7C** illustrates different patterns of miR expression were obtained in flow sorted B7H3+ or PSMA+ vesicle populations as compared to overall vesicle population.

[0060] **FIGs. 8A-H** illustrates detecting vesicles in a sample wherein the presence or level of the desired vesicles are assessed using a microsphere platform. **FIG. 8A** represents a schematic of isolating vesicles from plasma using a column based filtering method, wherein the isolated vesicles are subsequently assessed using a microsphere platform. **FIG. 8B** represents a schematic of compression of a membrane of a vesicle due to high-speed centrifugation, such as ultracentrifugation. **FIG. 8C** represents a schematic of detecting vesicles bound to microspheres using laser detection. **FIG. 8D** represents an example of detecting prostate derived vesicles bound to a substrate. The microvesicles are captured with capture agents specific to PCSA, PSMA or B7H3 tethered to

PREOMICS EXHIBIT 1002
Page 946 of 1861

the substrate. The so-captured vesicles are labeled with fluorescently labeled detection agents specific to CD9, CD63 and CD81. **FIG. 8E** illustrates correlation of CD9 positive vesicles detected using a microsphere platform (Y-axis) or flow cytometry (X-axis). To calculate median fluorescence intensity (MFIs), vesicles were captured with anti-CD9 antibodies tethered to microspheres and detected using fluorescently labeled detection antibodies specific to CD9, CD63 and CD81. **FIG. 8F** illustrates correlation of PSMA, PCSA or B7H3 positive vesicles detected using a microsphere platform (Y-axis) or BCA protein assay (X-axis). To calculate MFIs, vesicles were captured with antibodies to B7H3, PSMA or PCSA tethered to microspheres and detected using fluorescently labeled detection antibodies specific to CD9, CD63 and CD81. **FIG. 8G** illustrates similar performance for detecting CD81 positive vesicles using a microsphere assay in a single-plex or multi-plex fashion. Vesicles were captured with anti-CD81 antibodies tethered to microspheres and detected using fluorescently labeled detection antibodies specific to CD9, CD63 and CD81. **FIG. 8H** illustrates similar performance for detecting B7H3, CD63, CD9 or EpCam positive vesicles using a microsphere assay in a single-plex or multi-plex fashion. Vesicles were captured with antibodies to B7H3, CD63, CD9 or EpCam tethered to microspheres and detected using fluorescently labeled detection antibodies specific to CD9, CD63 and CD81.

[0061] **FIG. 9A** illustrates the ability of a vesicle bio-signature to discriminate between normal prostate and PCa samples. Cancer markers included EpCam and B7H3. General vesicle markers included CD9, CD81 and CD63. Prostate specific markers included PCSA. PSMA can be used as well as PCSA. The test was found to be 98% sensitive and 95% specific for PCa vs normal samples. **FIG. 9B** illustrates mean fluorescence intensity (MFI) on the Y axis for vesicle markers of **FIG. 9A** in normal and prostate cancer patients.

[0062] **FIG. 10** is a schematic for a decision tree for a vesicle prostate cancer assay for determining whether a sample is positive for prostate cancer.

[0063] **FIG. 11** shows the results of a vesicle detection assay for prostate cancer following the decision tree versus detection using elevated PSA levels.

[0064] **FIG. 12** illustrates levels of miR-145 in vesicles isolated from control and PCa samples.

[0065] **FIGs. 13A-13E** illustrate the use of microRNA to identify false negatives from a vesicle-based diagnostic assay for prostate cancer. **FIG. 13A** illustrates a scheme for using miR analysis within vesicles to convert false negatives into true positives, thereby improving sensitivity. **FIG. 13B** illustrates a scheme for using miR analysis within vesicles to convert false positives into true negatives, thereby improving specificity. Normalized levels of miR-107 **(FIG. 13C)** and miR-141 **(FIG. 13D)** are shown on the Y axis for true positives (TP) called by the vesicle diagnostic assay, true negatives (TN) called by the vesicle diagnostic assay, false positives (FP) called by the vesicle diagnostic assay, and false negatives (FN) called by the vesicle diagnostic assay. miR-107 and miR-141 can be used in the schematic shown in **FIG. 13A** and **FIG. 13B. FIG. 13E** shows Taqman qRT-PCR verification of increased miR-107 in plasma cMVs of prostate cancer patients compared to patients without prostate cancer using a different sample cohort.

[0066] **FIGs. 14A-D** illustrate KRAS sequencing in a colorectal cancer (CRC) cell line and patient sample. Samples comprise genomic DNA obtained from the cell line **(FIG. 14B)** or from a tissue sample from the patient **(FIG. 14D),** or cDNA obtained from RNA payload within vesicles shed from the cell line **(FIG. 14A)** or from a plasma sample from the patient **(FIG. 14C).**

[0067] **FIGs. 15A-B** illustrate immunoprecipitation of microRNA from human plasma. **FIG. 15A** shows the mean quantity of miR-16 detected in various fractions of human plasma. "Beads" are the amount of miR-16 that

PREOMICS EXHIBIT 1002
Page 947 of 1861

co-immunoprecipitated using antibodies to Argonaute2 **(Ago2),** Apolipoprotein A1 (ApoAl), GW182, and an IgG control. "Dyna" refers to immunoprecipitation using Dynabead Protein G, whereas "Magna" refers to Magnabind Protein G beads. "Supernt" are the amount of miR-16 detected in the supernatant of the immunoprecipitation reactions. See Examples for details. **FIG. 15B** is the same as **FIG. 15A** except that miR-92a was detected.

[0068] **FIG. 16** illustrates flow sorting of complexes stained with PE labeled anti-PCSA antibodies and FITC labeled anti-Ago2 antibodies.

[0069] **FIGs. 17A-D** illustrate detection of microRNA in PCSA/Ago2 positive complexes in human plasma samples. The plasma samples were from subjects with prostate cancer (PrC) or normal controls (normal). **FIG. 17A** shows miR-22 copy number in total circulating microvesicle population from human plasma. **FIG. 17B** shows plasma-derived complexes were sorted using antibodies against PCSA and Argonaute 2 (Ago2). RNA was isolated and the copy number of miR-22 was determined in the population of PCSA/Ago2 double positive events. **FIG. 17C** shows the number of PCSA/Ago2 double positive events counted by flow cytometry for each plasma sample. **FIG. 17D** shows copy number of miR-22 divided by the total number of PCSA/Ago2 positive events for each plasma sample. This yields the copy number of miR-22 per PCSA/Ago2 double positive complex.

[0070] **FIGs. 18A-D** illustrate flow cytometry of circulating microvesicles (cMVs) stained with anti-CD9 and/or anti-PCSA. **FIG. 18A** illustrates analysis of plasma derived cMVs using labeled antibodies to CD9 and PCSA. **FIG. 18B** illustrates an enrichment of double positive CD9/PCSA cMVs following double immunoprecipitation with anti-CD9 and anti-PCSA. Compare the double positive population in region R7 between **FIG. 18A** and **FIG. 18B. FIG. 18C** illustrates analysis of plasma derived cMVs using labeled antibodies to PCSA. **FIG. 18D** illustrates an enrichment of PCSA positive events following a single immunoprecipitation using antibodies against PCSA. Compare the population in region R4 between **FIG. 18C** and **FIG. 18D.**

[0071] **FIGs. 19A-G** illustrate levels of miR-22 in various plasma fractions. **FIG. 19A** illustrates miR-22 copy number in unmodified plasma as determined by ABI Taqman detection kit (Assay ID# 000398). **FIG. 19B** illustrates miR-22 copy number in the total circulating microvesicle population concentrated from patient plasma as determined by ABI Taqman detection kit. **FIG. 19C** illustrates miR-22 copy number retained on an anti-PCSA column using starting material that was released from an anti-CD9 column. **FIG. 19D** illustrates copy number of miR-22 relative to the sample-matched PCSA MFI as determined using a bead based assay. The average PCSA MFI signal for cancer and normal input plasma used for double immunoprecipitation was 161.67 and 729.17, respectively. **FIG. 19E** illustrates copy number of miR-22 in input plasma. **FIG. 19F** illustrates copy number of miR-22 from cMVs retained on the anti-PCSA column from the input plasma in **FIG. 19E.** **FIG. 19G** illustrates copy number of miR-22 relative to the sample-matched PCSA MFI as determined using a bead based assay. The average PCSA MFI signal for cancer and normal plasma used for single IP was 69.17 and 526.5, respectively.

[0072] **FIGs. 20A-C** illustrate distinguishing PCa and normal (non PCa) samples using a score derived from levels of PCSA and PSMA proteins and miR-22 and let7a microRNAs associated with cMVs isolated from plasma. **FIG. 20A** shows a plot of the score calculated for normal and cancer samples. **FIG. 20B** shows the data of **FIG.20A** where the normals are separated into groups of normal (no prostate conditions), atypia,

PREOMICS EXHIBIT 1002
Page 948 of 1861

inflammation and high grade prostatic intraepithelial neoplasia (high grade PIN, or HGPIN), and the cancers are separated into groups identified for watchful waiting (WW) or cancer. **FIG. 20C** shows an ROC curve generated with the data. The AUC was 0.77.

[0073] **FIGs. 21A-B** show illustrative plots for differential expression of miR-920 **(FIG. 21A)** and miR-450a **(FIG. 21B)** in different sample populations. The samples comprised microRNA in PCSA expressing cMVs isolated from plasma. miR-920 is overexpressed in confounding diseases (i.e., high grade PIN ("hgpin") and inflammatory disease ("inflammation")) as compared to prostate cancer ("cancer") and normals ("normal"). miR-450a is down regulated in cancers as compared to the others.

[0074] **FIGs. 22A-F** illustrate dot plots of raw background subtracted fluorescence values of selected mRNAs from microarray profiling of vesicle mRNA payload levels. In each plot, the Y axis shows raw background subtracted fluorescence values (Raw BGsub Florescence). The X axis shows dot plots for four normal control plasmas and four plasmas from prostate cancer patients. The mRNAs shown are A2ML1 **(FIG. 22A),** GABARAPL2 **(FIG. 22B),** PTMA **(FIG. 22C),** RABAC1 **(FIG. 22D),** SOX1 **(FIG. 22E),** and ETFB **(FIG. 22F).**

[0075] **FIGs. 23A-23B** illustrate levels of miR-141 **(FIG. 23A)** and miR-375 **(FIG. 23B)** in vesicles isolated from nonrecurring prostate cancer and metastatic prostate cancer samples, as indicated on the X axis. miRs isolated from vesicles were detected using Taqman assays. P values are shown below the plot. The Y axis shows copy number of miRs detected.

[0076] **FIGs. 24A-24B** illustrate microRNA miR-497 to distinguish between lung cancer and normal (non-lung cancer) using patient blood samples. The Y-axis shows copy number of miR-497 in 0.1 ml of sample. In **FIG. 24A,** the horizontal line indicates a copy number of 1154 copies. In **FIG. 24B,** the horizontal line indicates a copy number of 1356. **FIG. 24C** is a receiver operating characteristic (ROC) curve for distinguishing non-small cell lung cancer and normal plasma samples by examining levels of miR-497 in circulating microvesicles (cMV). The data corresponds to **FIG. 24B.**

[0077] **FIG. 25A** is an electron micrograph of Vcap-derived microvesicles bound to a glass slide, **FIG. 25B** is a scanning electron micrograph of Vcap-derived microvesicles, and **FIG. 25C** is a scanning electron micrograph of Vcap microvesicles bound to a polystyrene bead coated with poly-L-lysine. **FIG. 25D** illustrates blood processing into plasma as specified in a sample collection protocol.

[0078] **FIGs. 26A-E** illustrate a microRNA functional assay. **FIG. 26A** shows a labeled synthetic RNA molecule **261-266** and a ribonucleoprotein complex containing a target microRNA **267** of interest. **FIG. 26B** demonstrates cleavage of the synthetic RNA molecule at the target recognition site **263** when recognized by the ribonucleoprotein complex **267,** thereby releasing the label **265-266. FIGs. 26C-E** illustrate input ribonucleoprotein complex from various sources.

[0079] **FIGs. 27A-B** show panels of vesicle markers for distinguishing prostate cancer. In **FIG.27A,** vesicles were captured using antibodies to mammaglobin, SIM2 and NK-2R, each tethered to different populations of microbeads. The captured vesicles were detected with PE-labeled anti MFG-E8 antibodies. **FIG. 27A** shows ROC curve generated by distinguishing 61 prostate cancer and 68 non-prostate cancer samples based on the levels of the detected vesicles. The AUC was 0.90. At the point indicated on the graph by the arrow, the sensitivity was 0.85 and the specificity was 0.84. In **FIG.27B,** vesicles were captured using antibodies to Integrin, NK-2R, and Gal3, each tethered to different populations of microbeads. The captured vesicles were

PREOMICS EXHIBIT 1002
Page 949 of 1861

detected with PE-labeled anti MFG-E8 antibodies. **FIG. 27B** shows ROC curve generated by distinguishing 61 prostate cancer and 32 benign prostate samples (e.g., men with BPH without high inflammation) based on the levels of the detected vesicles. The AUC was 0.84. At the point indicated on the graph by the arrow, the sensitivity was 0.82 and the specificity was 0.75.

[0080] **FIGs. 28A-G** show levels of miRs detected in microvesicles from plasma of patients in the indicated sample groups. In **FIGs. 28A-G,** the y-axis is the C_t value from RT-PCR measurements of the miRs, and the x-axis groups the miR levels in the following sample groups, from left to right: 1) prostate cancer; 2) high grade pin (HGPEN); 3) inflammation; and 4) benign prostate disorder (e.g., BPH). **FIG 28A** shows the levels of miR-614. **FIG 28B** shows the levels of miR-21 1. **FIG 28C** shows the levels of miR-136. **FIG 28D** shows the levels of miR-149. **FIG 28E** shows the levels of miR-221 *. **FIG 28F** shows the levels of miR-329. **FIG 28G** shows the levels of miR-26b.

[0081] **FIGs. 29A-B** show ROC curves demonstrating the ability of different vesicle capture and detection agents to distinguish prostate cancer. In **FIG. 29A,** the capture agents recognized AURKB, A33, CD63, Gro-alpha, and Integrin, and the detectors recognized MUC2, PCSA, and CD81 . The AUC of the ROC curve was 0.8306, compared to only 0.59 for PSA. At the indicated point on the outermost ROC curve representing the vesicle markers, the sensitivity was 0.815 and the specificity was 0.737. In **FIG. 29B,** the capture agents recognized AURKB, CD63, FLNA, A33, Gro-alpha, Integrin, CD24, SSX2, and SIM2, and the detectors recognized MUC2, PCSA, CD81, MFG-E8, and EpCam. In this sample group, the AUC of the ROC curve was 0.835, compared to only 0.60 for PSA. At the indicated point on the outermost ROC curve representing the vesicle markers, the sensitivity was 0.823 and the specificity was 0.737.

[0082] **FIGs. 30A-C** demonstrate detection of cMVs that distinguish prostate cancer in plasma samples. Vesicles were captured with bead-tethered antibodies specific to PCSA, PSMA, or B7H3. The captured cMVs were labeled with PE-labeled antibodies to PSMA, PCSA, B7H3, or the tetraspanins CD9, CD63, and CD81. Results are shown in **FIG. 30A** for PCSA capture, **FIG. 30B** for PSMA capture, and **FIG. 30C** for B7H3 capture. In the figures, the Y-axis shows the average median fluorescence intensity (MFI) of the detected antibodies. Samples as shown on the X-axis included PCa positive pools ("1 Pos Pool"), negative control pools from patients without PCa ("2 Neg Pool"), and a control blank ("Blank"). The detection agents as indicated on the X-axis include labeled antibodies to PSMA, PCSA or B7H3 individually, a cocktail of the antibodies to PSMA, PCSA, B7H3 ("cocktail"), or a cocktail of antibodies to the tetraspanins CD9, CD63, and CD81 ("VI - tets").

[0083] **FIGs. 31A-F** show ROC curves demonstrating the ability of 3-marker panel vesicle capture and detection agents to distinguish prostate cancer. Illustrative results for distinguishing prostate cancer (PCa+) samples from all other samples (PCA-) (see **Table 53)** using 3-marker combinations are shown. The dark grey line (more jagged line to the left) corresponds to resubstitution performance and the smoother black line was generated using 10-fold cross-validation. ROC curves are shown generated using diagonal linear discriminant analysis **(FIG. 31A;** resubstitution AUC = 0.87; cross validation AUC = 0.86), linear discriminant analysis **(FIG. 31B;** resubstitution AUC = 0.87; cross validation AUC = 0.86), support vector machine **(FIG. 31C;** resubstitution AUC = 0.87; cross validation AUC = 0.86), tree-based gradient boosting **(FIG. 31D;** resubstitution AUC = 0.89; cross validation AUC = 0.84), lasso **(FIG. 31E;** resubstitution AUC = 0.87; cross

PREOMICS EXHIBIT 1002
Page 950 of 1861

validation AUC = 0.86), and neural network (**FIG. 31F;** resubstitution AUC = 0.87; cross validation AUC = 0.72).

[0084] **FIGs. 32A-C** illustrate the performance of a three marker panel consisting of the following markers: 1) Epcam detector - MMP7 capture; 2) PCSA detector - MMP7 capture; 3) Epcam detector - BCNP capture. The sample cohort was a restricted set wherein patients were age<75, serum PSA<10 ng/ml and no previous biopsy (N=127). An ROC curve generated using a diagonal linear discriminant analysis in this setting is shown in **FIG. 32A.** In the figure, the arrow indicates the threshold point along the curve where sensitivity equals 90% and specificity equals 80%. Another view of this threshold is shown in **FIG. 32B,** which shows the distribution of PCA+ and PCA- samples falling on either side of the indicated threshold line. The individual contribution of the Epcam detector - MMP7 capture marker is shown in **FIG. 32C.** "PCA, Current Biopsy" refers to men who had a first positive biopsy, whereas "PCA, Previous Biopsy" refers to the watchful waiting cohort.

[0085] **FIGs. 33A-B** show ROC curves demonstrating the ability of different vesicle capture and detection agents to distinguish prostate cancer. The performance of a 5-marker panel was determined in two settings using a linear discriminant analysis and 10-fold cross-validation or re-substitution methodology. ROC curves for the Model A setting (i.e., all PCa versus all other patient samples) are shown in **FIG. 33A.** The marker panel in this setting consisted of: 1) Epcam detector - MMP7 capture; 2) PCSA detector - MMP7 capture; 3) Epcam detector - BCNP capture; 4) PCSA detector - AdamlO capture; and 5) PCSA detector - KLK2 capture. In **FIG. 33A,** the upper more jagged line corresponds to the re-substitution method. The AUC was 0.90. Using cross-validation, the calculated AUC was 0.87. At the point indicated by the solid arrow, the model using cross-validation achieved 92% sensitivity and 50% specificity. At the point indicated by the solid arrow, the model using cross-validation achieved 82% sensitivity and 80% specificity. ROC curves for the Model C setting (i.e., restricted sample set as described below for **Table 53**) are shown in **FIG. 33B.** The marker panel in this setting consisted of: 1) Epcam detector - MMP7 capture; 2) PCSA detector - MMP7 capture; 3) Epcam detector - BCNP capture; 4) PCSA detector - AdamlO capture; and 5) CD81 detector - MMP7 capture. In **FIG. 33B,** the upper more jagged line corresponds to the re-substitution method. The AUC was 0.91 . Using cross-validation, the calculated AUC was 0.89. At the point indicated by the arrow, the cross-validation model achieved 95% sensitivity and 60% specificity.

[0086] **FIGs. 34A-D** shows levels of microRNA species in PCSA+ circulating microvesicles from the plasma of men with prostate cancer and benign prostate disorders. In **FIG. 34A,** the Ct from the Exiqon cards for miR-1974 (which overlaps a mitochondrial tRNA) is shown in the various pools. The prostate cancer samples had higher levels of this miR than other samples. **FIG. 34B** shows the copy number of the miR in the pools as measured by taqman analysis using an ABI 7900. In **FIG. 34C,** the Ct from the Exiqon cards for miR-320b is shown in the various pools. The prostate cancer samples had lower levels of this miR than other samples. **FIG. 34D** shows the copy number of miR-320b in the pools as measured by taqman analysis using an ABI 7900.

[0087] **FIG. 35** shows detection of a standard curve for a synthetic miR16 standard (10^6 - 10^1) and detection of miR16 in triplicate from a human plasma sample. As indicated by the legend, the data was taken from a Fluidigm Biomark (Fluidigm Corporation, South San Francisco, CA) using 48.48 Dynamic Array™ IFCs, 96.96 Dynamic Array™ IFCs, or with an ABI 7900HT Taqman assay (Applied Biosystems, Foster City, CA). All levels were determined under multiplex conditions.

PREOMICS EXHIBIT 1002
Page 951 of 1861

[0088] **FIGs. 36A-D** shows analysis of cMVs from plasma of prostate cancer and benign controls (i.e., non-prostate cancer) men using flow cytometry. cMVs in the plasma samples were first gated after labeling with a cocktail of labeled anti-tetraspanins antibodies (CD9, CD63, CD81). The identified cMVs were next labeled with PE-labeled anti-MMP7 antibodies and FITC-labeled anti-EpCAM antibodies. **FIG. 36** shows illustrative results for two prostate cancers **(FIG. 36C-D)** and two controls **(FIG. 36A-B).** The Y-axis indicates the detected levels of MMP7 and the X-axis indicates the detected levels of EpCAM.

[0089] **FIGs. 37A-G** show levels of alkaline phosphatase (intestinal) **(FIG. 37A),** CD-56 **(FIG. 37B),** CD-3 zeta **(FIG. 37C),** maplb **(FIG. 37D),** 14.3.3 pan **(FIG. 37E),** filamin **(FIG. 37F),** and thrombospondin **(FIG. 37G)** associated with microvesicles from plasma of normal (non-cancer) control individuals, breast cancer patients, brain cancer patients, lung cancer patients, colorectal cancer patients, colon adenoma patients, BPH patients (benign), inflamed prostate patients (inflammation), HGPIN patients, and prostate cancer patients, as indicated in the figures. Vesicles were concentrated then incubated with antibody arrays. Vesicles bound to antibodies to various proteins were fluorescently detected.

[0090] **FIGs. 38A-F** show results of immunoprecipitation of CD81, Ago2, IgG and BrdU. Precipitates were analyzed for the presence of microRNAs including let-7a **(FIG. 38A** and **FIG. 38B),** miR-16 **(FIG. 38C** and **FIG. 38D)** and mir-45 1 **(FIG. 38E** and **FIG. 38F).** The miRNAs were evaluated using ABI miRNA assays as follows: Hsa-Let-7a, Assay ID 377, Hsa-miR-16 Assay, Assay ID 391 and miR-45 1, Assay ID 1141 .

[0091] **FIGs. 39A-C** show the results of an Ago2 ELISA with lysed or intact concentrated plasma cMVs. **FIG. 39A** shows recombinant Ago2 detection in a plate-based ELISA in PBS (no lysis) or lysis buffer (lysed cMV). **FIG. 39B** shows the average OD 450 nm for endogenous Ago2 for concentrated plasma (cMV), intact and lysed. **FIG. 39C** shows estimated endogenous Ago2 (ng/mL) in concentrated plasma (cMV), intact or lysed.

[0092] **FIGs. 40A-B** show Argonaute 2 expression in a prostate cancer positive pool and a prostate cancer negative pool. **FIG. 40A** shows titration of blocking agent F127 in Ago2 plate based ELISA using sample and detector diluent 1%BSA+1%F68. **FIG. 40A** shows titration of blocking agent F127 in Ago2 plate based ELISA using sample and detector diluent 1%BSA+1%F127.

[0093] **FIG. 41** shows an example of using the protocol to detect cMVs from the peripheral blood of prostate cancer and normal patients. The cMVs were detected using Anti-MMP7-FITC antibody conjugate (Millipore anti-MMP7 monoclonal antibody 7B2). The plot shows the frequency of events detected versus concentration of the detection antibody.

[0094] **FIGs. 42A-J** illustrate flow sorting of vesicles and detection of miRs. cMV were stained for proteins associated with membranes such as tetraspanins (CD9, CD63, CD81), Ago2 and/or GW182 using a Beckman Coulter MoFlo XDP. See **Example 31** for general methodology. The flow cytometry methodology is outlined in **FIG. 42A. FIG. 42B** illustrates plasma concentrate from normal, prostate, and bladder cancer patients flow sorted for Tetraspanin (Tet)+/Ago2+/GW1 82- or Tet+/Ago2+/GW182+. **FIGs. 42C-E** show the levels of miR-22 detected in sample pools from the indicated fractions from the flow analysis shown in **FIG. 42B. FIG. 42C** shows the miR-22 level in the various samples in the unsorted plasma concentrate, which is the input to the flow sort. **FIG. 42D** shows the miR-22 level in the various samples in the Ago2+ Tet+ GW1 82- sorted population. **FIG. 42D** shows the miR-22 level in the various samples in the Ago2+ Tet+ GW1 82+ sorted population. Next, plasma concentrate from normal and various cancer patients was sorted for Tet+/Ago2+, Tet+/Ago2-, Tet-/Ago+. **FIG. 42F** illustrates sorting gates used to capture various cMV populations from the indicated samples.

PREOMICS EXHIBIT 1002
Page 952 of 1861

FIGs. 42G-I illustrate flow events detected in various samples for the indicated cMV populations. **FIG. 42G** shows the vesicles detected using tetraspanin detectors, which will detect all cMVs in the sample. **FIG. 42H** shows the vesicles detected using Ago2 detectors. **FIG. 421** shows the vesicles detected using both Tetraspanin and Ago2 detectors. RNA was extracted from concentrate and sorted populations and miRs were evaluated. **FIG. 42J** shows the relative copy number of the indicated miRs per vesicles detected in the 10 PCa samples relative to the 6 normal control samples. In **FIG. 42J,** the sorted vesicle populations are indicated along the x-axis as follows: **b)** Tet+Ago2+ **c)** Tet- Ago2+ **d)** Tet+ Ago2- **e)** input concentrate (not enriched).

[0095] **FIGs. 43A-G** illustrate association of GW1 82 with circulating microvesicles and Ago2 in human bodily fluids. **FIG. 43A** shows Western blot analysis for Ago2 in Dul45 lysate and purified VCaP exosomes. **FIG. 43B** shows immunoprecipitation of GW1 82 from human plasma. These data demonstrate co-immunoprecipitation of Ago2 with GW1 82 by Western blot. **FIGs. 43C-D** illustrate immunoprecipitation (IP) of microRNA from human peripheral blood. Anti-AG02 (abeam, ab571 13, lot GR291 17-1), anti-GW1 82 (Bethyl Labs, A302-330A) and anti-IgG (Santa Cruz sc-2025) capture antibodies were conjugated to Magnabind protein G beads (Thermo Scientific Cat. # 21349). Conjugated beads were incubated with human plasma. RNA was isolated and screened for select microRNAs (miR-16 and miR-92a) using ABI Taqman detection kits (ABI_391 and ABI_43 1), respectively. RNA was quantified against synthetic standards and normalized to IgG control. **FIG. 43C** shows levels of miR-92a and **FIG. 43D** shows levels of miR-16 detected. **FIGs. 43E-F** illustrate a sandwich ELISA demonstrating association of GW1 82 with Ago2 in human plasma. **FIG. 43E** shows titration of sample input using purified microvesicles and raw plasma by plate-based ELISA using anti-GW182 as a capture (GW1 82 (Bethyl Labs, A302-330A) and biotinylated anti-Ago2 (abeam, ab571 13, lot GR291 17-1) as a detector. The signal shown is normalized to no sample (NS) control. **FIG. 43F** shows a survey of seven patient samples, demonstrating detection of GW1 82:Ago2 binding in human plasma from different patients. The signal shown is normalized to no sample (NS) control. **FIG. 43G** illustrates association of GW1 82 with Argonautes in human urine. The relationship between human GW1 82 and the Argonaute family of proteins was investigated in urine using a microbead detection system. Particles were captured with anti-GW1 82 antibody followed by detection with anti-pan Argonaute antibody using five patient urine samples. Conditions included raw vs cell + hard spun urine.

[0096] **FIG. 44** illustrates the use of an anti-EpCAM aptamer (Aptamer 4; SEQ ID NO. 1) to detect a microvesicle population. Vesicles in patient plasma samples were captured using bead-conjugated antibodies to the indicated microvesicle surface antigens. Fluorescently labeled Aptamer 4 was used as a detector in the microbead assay. The figure shows average median fluorescence values (MFI values) for three prostate cancer (C1-C3) and three normal samples (N1-N3) in each plot. In each plot, the samples from left to right are ordered as: Cl, C2, C3, N1, N2, N3.

DETAILED DESCRIPTION OF THE INVENTION

[0097] Disclosed herein are methods and systems for characterizing a phenotype of a biological sample, e.g., a sample from a cell culture, an organism, or a subject. The phenotype can be characterized by assessing one or more biomarkers. The biomarkers can be associated with a vesicle or vesicle population, either presented vesicle surface antigens or vesicle payload. As used herein, vesicle payload comprises entities encapsulated within a vesicle. Vesicle associated biomarkers can comprise both membrane bound and soluble biomarkers. The biomarkers can also be circulating biomarkers, such as nucleic acids (e.g., microRNA) or protein/polypeptide, or

PREOMICS EXHIBIT 1002
Page 953 of 1861

functional fragments thereof, assessed in a bodily fluid. Unless otherwise specified, the terms "purified" or "isolated" as used herein in reference to vesicles or biomarker components mean partial or complete purification or isolation of such components from a cell or organism. Furthermore, unless otherwise specified, reference to vesicle isolation using a binding agent includes binding a vesicle with the binding agent whether or not such binding results in complete isolation of the vesicle apart from other biological entities in the starting material.

[0098] A method of characterizing a phenotype by analyzing a circulating biomarker, e.g., a nucleic acid biomarker, is depicted in scheme **6100A** of **FIG. 1A,** as a non-limiting illustrative example. In a first step **6101,** a biological sample is obtained, e.g., a bodily fluid, tissue sample or cell culture. Nucleic acids are isolated from the sample **6103.** The nucleic acid can be DNA or RNA, e.g., microRNA. Assessment of such nucleic acids can provide a biosignature for a phenotype. By sampling the nucleic acids associated with target phenotype (e.g., disease versus healthy, pre- and post-treatment), one or more nucleic acid markers that are indicative of the phenotype can be determined. Various aspects of the present invention are directed to biosignatures determined by assessing one or more nucleic acid molecules (e.g., microRNA) present in the sample **6105,** where the biosignature corresponds to a predetermined phenotype **6107. FIG. IB** illustrates a scheme **6100B** of using vesicles to determine a biosignature and/or characterize a phenotype. In one example, a biological sample is obtained **6102,** and one or more vesicles of interest, e.g., all vesicles, or vesicles from a particular cell-of-origin and/or vesicles associated with a particular disease state, are isolated from the sample **6104.** The vesicles can be analyzed **6106** by characterizing surface antigens associated with the vesicles and/or determining the presence or levels of components present within the vesicles ("payload"). Unless specified otherwise, the term "antigen" as used herein refers generally to a biomarker that can be bound by a binding agent, whether the binding agent is an antibody, aptamer, lectin, or other binding agent for the biomarker and regardless of whether such biomarker illicits an immune response in a host. Vesicle payload including without limitation protein, including peptides and polypeptides, nucleic acids such as DNA and RNAs, lipids and/or carbohydrates. RNA payload includes messenger RNA (mRNA) and microRNA (also referred to herein as miRNA or miR). A phenotype is characterized based on the biosignature of the vesicles **6108.** In another illustrative method of the invention, schemes **6100A** and **6100B** are performed together to characterize a phenotype. In such a scheme, vesicles and nucleic acids, e.g., microRNA, are assessed, thereby characterizing the phenotype.

[0099] According to the methods of the invention, multiple biomarkers can be assessed sequentially or concurrently to characterize a phenotype. For example, a subpopulation of vesicles can be assessed by concurrently detecting two vesicle surface antigens, e.g., using binding agents to both capture and detect vesicles. In another example, a subpopulation of vesicles can be assessed by sequentially detecting a vesicle surface antigen, e.g., to capture vesicles, and then the captured vesicles can be assessed for payload such as mRNA, microRNA or soluble protein. In some embodiments, characterizing a phenotype comprises both the concurrent assessment of one or more biomarker and sequential assessment of one or more other biomarker. As a non-limiting example, a vesicle subpopulation that is detecting using binding agents to more than one surface antigen can be sorted, and then payload can be assessed, e.g., one or more miRs. One of skill will recognize that many variations of sequential or concurrent assessment of biomarkers can be used to characterize a phenotype.

[00100] In another related aspect, methods are provided herein for the discovery of biomarkers comprising assessing vesicle surface markers or payload markers in one sample and comparing the markers to another sample. Markers that distinguish between the samples can be used as biomarkers according to the invention.

PREOMICS EXHIBIT 1002
Page 954 of 1861

Such samples can be from a subject or group of subjects. For example, the groups can be, e.g., diseased versus normal (e.g., non-diseased), known responders and non-responders to a given treatment for a given disease or disorder. Biomarkers discovered to distinguish the known responders and non-responders provide a biosignature of whether a subject is likely to respond to a treatment such as a therapeutic agent, e.g., a drug or biologic.

Phenotypes

[00101] Disclosed herein are products and processes for characterizing a phenotype of an individual by analyzing a vesicle such as a membrane vesicle. A phenotype can be any observable characteristic or trait of a subject, such as a disease or condition, a disease stage or condition stage, susceptibility to a disease or condition, prognosis of a disease stage or condition, a physiological state, or response to therapeutics. A phenotype can result from a subject's gene expression as well as the influence of environmental factors and the interactions between the two, as well as from epigenetic modifications to nucleic acid sequences.

[00102] A phenotype in a subject can be characterized by obtaining a biological sample from a subject and analyzing one or more vesicles from the sample. For example, characterizing a phenotype for a subject or individual may include detecting a disease or condition (including pre-symptomatic early stage detecting), determining the prognosis, diagnosis, or theranosis of a disease or condition, or determining the stage or progression of a disease or condition. Characterizing a phenotype can also include identifying appropriate treatments or treatment efficacy for specific diseases, conditions, disease stages and condition stages, predictions and likelihood analysis of disease progression, particularly disease recurrence, metastatic spread or disease relapse. A phenotype can also be a clinically distinct type or subtype of a condition or disease, such as a cancer or tumor. Phenotype determination can also be a determination of a physiological condition, or an assessment of organ distress or organ rejection, such as post-transplantation. The products and processes described herein allow assessment of a subject on an individual basis, which can provide benefits of more efficient and economical decisions in treatment.

[00103] In an aspect, the invention relates to the analysis of a biological sample to identify a biosignature to predict whether a subject is likely to respond to a treatment for a disease or disorder. Characterizating a phenotype includes predicting the responder / non-responder status of the subject, wherein a responder responds to a treatment for a disease and a non-responder does not respond to the treatment. Vesicles can be analyzed in the subject and compared to vesicle analysis of previous subjects that were known to respond or not to a treatment. If the vesicle biosignature in a subject more closely aligns with that of previous subjects that were known to respond to the treatment, the subject can be characterized, or predicted, as a responder to the treatment. Similarly, if the vesicle biosignature in the subject more closely aligns with that of previous subjects that did not respond to the treatment, the subject can be characterized, or predicted as a non-responder to the treatment. The treatment can be for any appropriate disease, disorder or other condition. The method can be used in any disease setting where a vesicle biosignature that correlates with responder / non-responder status is known.

[00104] The term "phenotype" as used herein can mean any trait or characteristic that is attributed to a vesicle biosignature that is identified using methods of the invention. For example, a phenotype can be the identification of a subject as likely to respond to a treatment, or more broadly, it can be a diagnostic, prognostic or theranostic determination based on a characterized biosignature for a sample obtained from a subject.

PREOMICS EXHIBIT 1002
Page 955 of 1861

[00105] In some embodiments, the phenotype comprises a disease or condition such as those listed in **Table 1.** For example, the phenotype can comprise the presence of or likelihood of developing a tumor, neoplasm, or cancer. A cancer detected or assessed by products or processes described herein includes, but is not limited to, breast cancer, ovarian cancer, lung cancer, colon cancer, hyperplastic polyp, adenoma, colorectal cancer, high grade dysplasia, low grade dysplasia, prostatic hyperplasia, prostate cancer, melanoma, pancreatic cancer, brain cancer (such as a glioblastoma), hematological malignancy, hepatocellular carcinoma, cervical cancer, endometrial cancer, head and neck cancer, esophageal cancer, gastrointestinal stromal tumor (GIST), renal cell carcinoma (RCC) or gastric cancer. The colorectal cancer can be CRC Dukes B or Dukes C-D. The hematological malignancy can be B-Cell Chronic Lymphocytic Leukemia, B-Cell Lymphoma-DLBCL, B-Cell Lymphoma-DLBCL-germinal center-like, B-Cell Lymphoma-DLBCL-activated B-cell-like, and Burkitt's lymphoma.

[00106] The phenotype can be a premalignant condition, such as actinic keratosis, atrophic gastritis, leukoplakia, erythroplasia, Lymphomatoid Granulomatosis, preleukemia, fibrosis, cervical dysplasia, uterine cervical dysplasia, xeroderma pigmentosum, Barrett's Esophagus, colorectal polyp, or other abnormal tissue growth or lesion that is likely to develop into a malignant tumor. Transformative viral infections such as HIV and HPV also present phenotypes that can be assessed according to the invention.

[00107] The cancer characterized by the methods of the invention can comprise, without limitation, a carcinoma, a sarcoma, a lymphoma or leukemia, a germ cell tumor, a blastoma, or other cancers. Carcinomas include without limitation epithelial neoplasms, squamous cell neoplasms squamous cell carcinoma, basal cell neoplasms basal cell carcinoma, transitional cell papillomas and carcinomas, adenomas and adenocarcinomas (glands), adenoma, adenocarcinoma, linitis plastica insulinoma, glucagonoma, gastrinoma, vipoma, cholangiocarcinoma, hepatocellular carcinoma, adenoid cystic carcinoma, carcinoid tumor of appendix, prolactinoma, oncocytoma, hurthle cell adenoma, renal cell carcinoma, grawitz tumor, multiple endocrine adenomas, endometrioid adenoma, adnexal and skin appendage neoplasms, mucoepidermoid neoplasms, cystic, mucinous and serous neoplasms, cystadenoma, pseudomyxoma peritonei, ductal, lobular and medullary neoplasms, acinar cell neoplasms, complex epithelial neoplasms, warthin's tumor, thymoma, specialized gonadal neoplasms, sex cord stromal tumor, thecoma, granulosa cell tumor, arrhenoblastoma, Sertoli leydig cell tumor, glomus tumors, paraganglioma, pheochromocytoma, glomus tumor, nevi and melanomas, melanocytic nevus, malignant melanoma, melanoma, nodular melanoma, dysplastic nevus, lentigo maligna melanoma, superficial spreading melanoma, and malignant acral lentiginous melanoma. Sarcoma includes without limitation Askin's tumor, botryodies, chondrosarcoma, Ewing's sarcoma, malignant hemangio endothelioma, malignant schwannoma, osteosarcoma, soft tissue sarcomas including: alveolar soft part sarcoma, angiosarcoma, cystosarcoma phyllodes, dermatofibrosarcoma, desmoid tumor, desmoplastic small round cell tumor, epithelioid sarcoma, extraskeletal chondrosarcoma, extraskeletal osteosarcoma, fibrosarcoma, hemangiopericytoma, hemangiosarcoma, kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, lymphosarcoma, malignant fibrous histiocytoma, neurofibrosarcoma, rhabdomyosarcoma, and synovialsarcoma. Lymphoma and leukemia include without limitation chronic lymphocytic leukemia/small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma (such as Waldenstrom macroglobulinemia), splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, monoclonal immunoglobulin deposition diseases, heavy chain diseases, extranodal marginal zone B cell lymphoma, also called malt lymphoma, nodal

PREOMICS EXHIBIT 1002
Page 956 of 1861

marginal zone B cell lymphoma (nmzl), follicular lymphoma, mantle cell lymphoma, diffuse large B cell lymphoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, burkitt lymphoma/leukemia, T cell prolymphocyte leukemia, T cell large granular lymphocytic leukemia, aggressive NK cell leukemia, adult T cell leukemia/lymphoma, extranodal NK/T cell lymphoma, nasal type, enteropathy-type T cell lymphoma, hepatosplenic T cell lymphoma, blastic NK cell lymphoma, mycosis fungoides / sezary syndrome, primary cutaneous CD30-positive T cell lymphoproliferative disorders, primary cutaneous anaplastic large cell lymphoma, lymphomatoid papulosis, angioimmunoblastic T cell lymphoma, peripheral T cell lymphoma, unspecified, anaplastic large cell lymphoma, classical hodgkin lymphomas (nodular sclerosis, mixed cellularity, lymphocyte-rich, lymphocyte depleted or not depleted), and nodular lymphocyte-predominant hodgkin lymphoma. Germ cell tumors include without limitation germinoma, dysgerminoma, seminoma, nongerminomatous germ cell tumor, embryonal carcinoma, endodermal sinus turmor, choriocarcinoma, teratoma, polyembryoma, and gonadoblastoma. Blastoma includes without limitation nephroblastoma, meduUoblastoma, and retinoblastoma. Other cancers include without limitation labial carcinoma, larynx carcinoma, hypopharynx carcinoma, tongue carcinoma, salivary gland carcinoma, gastric carcinoma, adenocarcinoma, thyroid cancer (medullary and papillary thyroid carcinoma), renal carcinoma, kidney parenchyma carcinoma, cervix carcinoma, uterine corpus carcinoma, endometrium carcinoma, chorion carcinoma, testis carcinoma, urinary carcinoma, melanoma, brain tumors such as glioblastoma, astrocytoma, meningioma, meduUoblastoma and peripheral neuroectodermal tumors, gall bladder carcinoma, bronchial carcinoma, multiple myeloma, basalioma, teratoma, retinoblastoma, choroidea melanoma, seminoma, rhabdomyosarcoma, craniopharyngeoma, osteosarcoma, chondrosarcoma, myosarcoma, liposarcoma, fibrosarcoma, Ewing sarcoma, and plasmocytoma.

[00108] In a further embodiment, the cancer under analysis may be a lung cancer including non-small cell lung cancer and small cell lung cancer (including small cell carcinoma (oat cell cancer), mixed small cell/large cell carcinoma, and combined small cell carcinoma), colon cancer, breast cancer, prostate cancer, liver cancer, pancreas cancer, brain cancer, kidney cancer, ovarian cancer, stomach cancer, skin cancer, bone cancer, gastric cancer, breast cancer, pancreatic cancer, glioma, glioblastoma, hepatocellular carcinoma, papillary renal carcinoma, head and neck squamous cell carcinoma, leukemia, lymphoma, myeloma, or a solid tumor.

[00109] In embodiments, the cancer comprises an acute lymphoblastic leukemia; acute myeloid leukemia; adrenocortical carcinoma; AIDS-related cancers; AIDS-related lymphoma; anal cancer; appendix cancer; astrocytomas; atypical teratoid/rhabdoid tumor; basal cell carcinoma; bladder cancer; brain stem glioma; brain tumor (including brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, astrocytomas, craniopharyngioma, ependymoblastoma, ependymoma, meduUoblastoma, meduUoepithelioma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma); breast cancer; bronchial tumors; Burkitt lymphoma; cancer of unknown primary site; carcinoid tumor; carcinoma of unknown primary site; central nervous system atypical teratoid/rhabdoid tumor; central nervous system embryonal tumors; cervical cancer; childhood cancers; chordoma; chronic lymphocytic leukemia; chronic myelogenous leukemia; chronic myeloproliferative disorders; colon cancer; colorectal cancer; craniopharyngioma; cutaneous T-cell lymphoma; endocrine pancreas islet cell tumors; endometrial cancer; ependymoblastoma; ependymoma; esophageal cancer; esthesioneuroblastoma; Ewing sarcoma; extracranial germ cell tumor; extragonadal germ cell tumor; extrahepatic bile duct cancer;

PREOMICS EXHIBIT 1002
Page 957 of 1861

gallbladder cancer; gastric (stomach) cancer; gastrointestinal carcinoid tumor; gastrointestinal stromal cell tumor; gastrointestinal stromal tumor (GIST); gestational trophoblastic tumor; glioma; hairy cell leukemia; head and neck cancer; heart cancer; Hodgkin lymphoma; hypopharyngeal cancer; intraocular melanoma; islet cell tumors; Kaposi sarcoma; kidney cancer; Langerhans cell histiocytosis; laryngeal cancer; lip cancer; liver cancer; malignant fibrous histiocytoma bone cancer; medulloblastoma; medulloepithelioma; melanoma; Merkel cell carcinoma; Merkel cell skin carcinoma; mesothelioma; metastatic squamous neck cancer with occult primary; mouth cancer; multiple endocrine neoplasia syndromes; multiple myeloma; multiple myeloma/plasma cell neoplasm; mycosis fungoides; myelodysplastic syndromes; myeloproliferative neoplasms; nasal cavity cancer; nasopharyngeal cancer; neuroblastoma; Non-Hodgkin lymphoma; nonmelanoma skin cancer; non-small cell lung cancer; oral cancer; oral cavity cancer; oropharyngeal cancer; osteosarcoma; other brain and spinal cord tumors; ovarian cancer; ovarian epithelial cancer; ovarian germ cell tumor; ovarian low malignant potential tumor; pancreatic cancer; papillomatosis; paranasal sinus cancer; parathyroid cancer; pelvic cancer; penile cancer; pharyngeal cancer; pineal parenchymal tumors of intermediate differentiation; pineoblastoma; pituitary tumor; plasma cell neoplasm/multiple myeloma; pleuropulmonary blastoma; primary central nervous system (CNS) lymphoma; primary hepatocellular liver cancer; prostate cancer; rectal cancer; renal cancer; renal cell (kidney) cancer; renal cell cancer; respiratory tract cancer; retinoblastoma; rhabdomyosarcoma; salivary gland cancer; Sezary syndrome; small cell lung cancer; small intestine cancer; soft tissue sarcoma; squamous cell carcinoma; squamous neck cancer; stomach (gastric) cancer; supratentorial primitive neuroectodermal tumors; T-cell lymphoma; testicular cancer; throat cancer; thymic carcinoma; thymoma; thyroid cancer; transitional cell cancer; transitional cell cancer of the renal pelvis and ureter; trophoblastic tumor; ureter cancer; urethral cancer; uterine cancer; uterine sarcoma; vaginal cancer; vulvar cancer; Waldenstrom macroglobulinemia; or Wilm's tumor. The methods of the invention can be used to characterize these and other cancers. Thus, characterizing a phenotype can be providing a diagnosis, prognosis or theranosis of one of the cancers disclosed herein.

[00110] The phenotype can also be an inflammatory disease, immune disease, or autoimmune disease. For example, the disease may be inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), pelvic inflammation, vasculitis, psoriasis, diabetes, autoimmune hepatitis, Multiple Sclerosis, Myasthenia Gravis, Type I diabetes, Rheumatoid Arthritis, Psoriasis, Systemic Lupus Erythematosis (SLE), Hashimoto's Thyroiditis, Grave's disease, Ankylosing Spondylitis Sjogrens Disease, CREST syndrome, Scleroderma, Rheumatic Disease, organ rejection, Primary Sclerosing Cholangitis, or sepsis.

[00111] The phenotype can also comprise a cardiovascular disease, such as atherosclerosis, congestive heart failure, vulnerable plaque, stroke, or ischemia. The cardiovascular disease or condition can be high blood pressure, stenosis, vessel occlusion or a thrombotic event.

[00112] The phenotype can also comprise a neurological disease, such as Multiple Sclerosis (MS), Parkinson's Disease (PD), Alzheimer's Disease (AD), schizophrenia, bipolar disorder, depression, autism, Prion Disease, Pick's disease, dementia, Huntington disease (HD), Down's syndrome, cerebrovascular disease, Rasmussen's encephalitis, viral meningitis, neurospsychiatric systemic lupus erythematosus (NPSLE), amyotrophic lateral sclerosis, Creutzfeldt-Jacob disease, Gerstmann-Straussler-Scheinker disease, transmissible spongiform encephalopathy, ischemic reperfusion damage (e.g. stroke), brain trauma, microbial infection, or chronic fatigue syndrome. The phenotype may also be a condition such as fibromyalgia, chronic neuropathic pain, or peripheral neuropathic pain.

PREOMICS EXHIBIT 1002
Page 958 of 1861

[00113] The phenotype may also comprise an infectious disease, such as a bacterial, viral or yeast infection. For example, the disease or condition may be Whipple's Disease, Prion Disease, cirrhosis, methicillin-resistant staphylococcus aureus, HIV, hepatitis, syphilis, meningitis, malaria, tuberculosis, or influenza. Viral proteins, such as HIV or HCV-like particles can be assessed in a vesicle, to characterize a viral condition.

[00114] The phenotype can also comprise a perinatal or pregnancy related condition (e.g. preeclampsia or preterm birth), metabolic disease or condition, such as a metabolic disease or condition associated with iron metabolism. For example, hepcidin can be assayed in a vesicle to characterize an iron deficiency. The metabolic disease or condition can also be diabetes, inflammation, or a perinatal condition.

[00115] The methods of the invention can be used to characterize these and other diseases and disorders that can be assessed via a candidate biosignature comprising one or a plurality of biomarkers. Thus, characterizing a phenotype can be providing a diagnosis, prognosis or theranosis of one of the diseases and disorders disclosed herein.

[00116] In various embodiments of the invention, a biosignature for any of the conditions or diseases disclosed herein can comprise one or more biomarkers in one of several different categories of markers, wherein the categories include one or more of: 1) disease specific biomarkers; 2) cell- or tissue-specific biomarkers; 3) vesicle-specific markers (e.g., general vesicle biomarkers); 4. angiogenesis-specific biomarkers; and 5) immunomodulatory biomarkers. Examples of all such markers are disclosed herein and known to a person having ordinary skill in the art. Furthermore, a biomarker known in the art that is characterized to have a role in a particular disease or condition can be adapted for use as a target in compositions and methods of the invention. In further embodiments, such biomarkers can be all vesicle surface markers, or a combination of vesicle surface markers and vesicle payload markers (i.e., molecules enclosed by a vesicle). In addition, as noted herein, the biological sample assessed can be any biological fluid, or can comprise individual components present within such biological fluid (e.g., vesicles, nucleic acids, proteins, or complexes thereof).

Subject

[00117] One or more phenotypes of a subject can be determined by analyzing one or more vesicles, such as vesicles, in a biological sample obtained from the subject. A subject or patient can include, but is not limited to, mammals such as bovine, avian, canine, equine, feline, ovine, porcine, or primate animals (including humans and non-human primates). A subject can also include a mammal of importance due to being endangered, such as a Siberian tiger; or economic importance, such as an animal raised on a farm for consumption by humans, or an animal of social importance to humans, such as an animal kept as a pet or in a zoo. Examples of such animals include, but are not limited to, carnivores such as cats and dogs; swine including pigs, hogs and wild boars; ruminants or ungulates such as cattle, oxen, sheep, giraffes, deer, goats, bison, camels or horses. Also included are birds that are endangered or kept in zoos, as well as fowl and more particularly domesticated fowl, i.e. poultry, such as turkeys and chickens, ducks, geese, guinea fowl. Also included are domesticated swine and horses (including race horses). In addition, any animal species connected to commercial activities are also included such as those animals connected to agriculture and aquaculture and other activities in which disease monitoring, diagnosis, and therapy selection are routine practice in husbandry for economic productivity and/or safety of the food chain.

PREOMICS EXHIBIT 1002
Page 959 of 1861

[00118] The subject can have a pre-existing disease or condition, such as cancer. Alternatively, the subject may not have any known pre-existing condition. The subject may also be non-responsive to an existing or past treatment, such as a treatment for cancer.

Samples

[00119] The biological sample obtained from the subject can be any bodily or biological fluid. For example, the biological sample can be any biological fluid including but not limited to peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen (including prostatic fluid), Cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates or other lavage fluids. A biological sample may also include the blastocyl cavity, umbilical cord blood, or maternal circulation which may be of fetal or maternal origin. The biological sample may also be a tissue sample or biopsy from which vesicles and other circulating biomarkers may be obtained. For example, cells from the sample can be cultured and vesicles isolated from the culture (see **Examples).** In various embodiments, biomarkers and/or biosignatures disclosed herein can be assessed directly from such biological samples (e.g., identification of presence or levels of nucleic acid or polypeptide biomarkers or functional fragments thereof) using various methods, such as extraction of nucleic acid molecules from blood, plasma, serum or any of the foregoing biological samples, use of protein or antibody arrays to identify polypeptide (or functional fragment) biomarker(s), as well as other array, sequencing, PCR and proteomic techniques known in the art for identification and assessment of nucleic acid and polypeptide molecules. In addition, one or more components present in such samples can be first isolated or enriched and further processed to assess the presence or levels of selected biomarkers, e.g., to assess a given biosignature. For example, microvesicles can be isolated from a sample prior to profiling the microvesicles for protein and/or nucleic acid biomarkers.

[00120] **Table 1** lists illustrative examples of diseases, conditions, or biological states and a corresponding list of biological samples from which vesicles may be analyzed.

Table 1: Examples of Biological Samples for Vesicle Analysis for Various Diseases, Conditions, or Biological States

Illustrative Disease, Condition or Biological State	Illustrative Biological Samples
Cancers/neoplasms affecting the following tissue types/bodily systems: breast, lung, ovarian, colon, rectal, prostate, pancreatic, brain, bone, connective tissue, glands, skin, lymph, nervous system, endocrine, germ cell, genitourinary, hematologic/blood, bone marrow, muscle, eye, esophageal, fat tissue, thyroid, pituitary, spinal cord, bile duct, heart, gall bladder, bladder, testes, cervical, endometrial, renal, ovarian, digestive/gastrointestinal, stomach, head and neck, liver, leukemia, respiratory/thorasic, cancers of unknown primary (CUP)	Blood, serum, plasma, cerebrospinal fluid (CSF), urine, sputum, ascites, synovial fluid, semen, nipple aspirates, saliva, bronchoalveolar lavage fluid, tears, oropharyngeal washes, feces, peritoneal fluids, pleural effusion, sweat, tears, aqueous humor, pericardial fluid, lymph, chyme, chyle, bile, stool water, amniotic fluid, breast milk, pancreatic juice, cerumen, Cowper's fluid or pre-ejaculatory fluid, female ejaculate, interstitial fluid, menses, mucus, pus, sebum, vaginal lubrication, vomit
Neurodegenerative/neurological disorders: Parkinson's disease, Alzheimer's Disease and multiple sclerosis, Schizophrenia, and bipolar disorder, spasticity disorders, epilepsy	Blood, serum, plasma, CSF, urine

PREOMICS EXHIBIT 1002
Page 960 of 1861

Cardiovascular Disease: atherosclerosis, cardiomyopathy, endocarditis, vunerable plaques, infection	Blood, serum, plasma, CSF, urine
Stroke: ischemic, intracerebral hemorrhage, subarachnoid hemorrhage, transient ischemic attacks (TIA)	Blood, serum, plasma, CSF, urine
Pain disorders: peripheral neuropathic pain and chronic neuropathic pain, and fibromyalgia,	Blood, serum, plasma, CSF, urine
Autoimmune disease: systemic and localized diseases, rheumatic disease, Lupus, Sjogren's syndrome	Blood, serum, plasma, CSF, urine, synovial fluid
Digestive system abnormalities: Barrett's esophagus, irritable bowel syndrome, ulcerative colitis, Crohn's disease, Diverticulosis and Diverticulitis, Celiac Disease	Blood, serum, plasma, CSF, urine
Endocrine disorders: diabetes mellitus, various forms of Thyroiditis, adrenal disorders, pituitary disorders	Blood, serum, plasma, CSF, urine
Diseases and disorders of the skin: psoriasis	Blood, serum, plasma, CSF, urine, synovial fluid, tears
Urological disorders: benign prostatic hypertrophy (BPH), polycystic kidney disease, interstitial cystitis	Blood, serum, plasma, urine
Hepatic disease/injury: Cirrhosis, induced hepatotoxicity (due to exposure to natural or synthetic chemical sources)	Blood, serum, plasma, urine
Kidney disease/injury: acute, sub-acute, chronic conditions, Podocyte injury, focal segmental glomerulosclerosis	Blood, serum, plasma, urine
Endometriosis	Blood, serum, plasma, urine, vaginal fluids
Osteoporosis	Blood, serum, plasma, urine, synovial fluid
Pancreatitis	Blood, serum, plasma, urine, pancreatic juice
Asthma	Blood, serum, plasma, urine, sputum, bronchiolar lavage fluid
Allergies	Blood, serum, plasma, urine, sputum, bronchiolar lavage fluid
Prion-related diseases	Blood, serum, plasma, CSF, urine
Viral Infections: HIV/AIDS	Blood, serum, plasma, urine
Sepsis	Blood, serum, plasma, urine, tears, nasal lavage
Organ rejection/transplantation	Blood, serum, plasma, urine, various lavage fluids
Differentiating conditions: adenoma versus hyperplastic polyp, irritable bowel syndrome (IBS) versus normal, classifying Dukes stages A, B, C, and/or D of colon cancer, adenoma with low-grade hyperplasia versus high-grade hyperplasia, adenoma versus normal, colorectal cancer versus normal, IBS versus. ulcerative colitis (UC) versus Crohn's disease (CD),	Blood, serum, plasma, urine, sputum, feces, colonic lavage fluid
Pregnancy related physiological states, conditions, or affiliated diseases: genetic risk, adverse pregnancy outcomes	Maternal serum, plasma, amniotic fluid, cord blood

[00121] The methods of the invention can be used to characterize a phenotype using a blood sample or blood derivative. Blood derivatives include plasma and serum. Blood plasma is the liquid component of whole blood, and makes up approximately 55% of the total blood volume. It is composed primarily of water with small amounts of minerals, salts, ions, nutrients, and proteins in solution. In whole blood, red blood cells, leukocytes,

PREOMICS EXHIBIT 1002
Page 961 of 1861

and platelets are suspended within the plasma. Blood serum refers to blood plasma without fibrinogen or other clotting factors (i.e., whole blood minus both the cells and the clotting factors).

[00122] The biological sample may be obtained through a third party, such as a party not performing the analysis of the biomarkers, whether direct assessment of a biological sample or by profiling one or more vesicles obtained from the biological sample. For example, the sample may be obtained through a clinician, physician, or other health care manager of a subject from which the sample is derived. Alternatively, the biological sample may obtained by the same party analyzing the vesicle. In addition, biological samples be assayed, are archived (e.g., frozen) or ortherwise stored in under preservative conditions.

[00123] The volume of the biological sample used for biomarker analysis can be in the range of between 0.1-20 mL, such as less than about 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0.1 mL.

[00124] A sample of bodily fluid can be used as a sample for characterizing a phenotype. For example, biomarkers in the sample can be assessed to provide a diagnosis, prognosis and/or theranosis of a disease. The biomarkers can be circulating biomarkers, such as circulating proteins or nucleic acids. The biomarkers can also be associated with a vesicle or vesicle population. Methods of the invention can be applied to assess one or more vesicles, as well as one or more different vesicle populations that may be present in a biological sample or in a subject. Analysis of one or more biomarkers in a biological sample can be used to determine whether an additional biological sample should be obtained for analysis. For example, analysis of one or more vesicles in a sample of bodily fluid can aid in determining whether a tissue biopsy should be obtained.

[00125] A sample from a patient can be collected under conditions that preserve the circulating biomarkers and other entities of interest contained therein for subsequent analysis. In an embodiment, the samples are processed using one or more of CellSave Preservative Tubes (Veridex, North Raritan, NJ), PAXgene Blood DNA Tubes (QIAGEN GmbH, Germany), and RNAlater (QIAGEN GmbH, Germany).

[00126] CellSave Preservative Tubes (CellSave tubes) are sterile evacuated blood collection tubes. Each tube contains a solution that contains Na2EDTA and a cell preservative. The EDTA absorbs calcium ions, which can reduce or eliminate blood clotting. The preservative preserves the morphology and cell surface antigen expression of epithelial and other cells. The collection and processing can be performed as described in a protocol provided by the manufacturer. Each tube is evacuated to withdraw venous whole blood following standard phlebotomy procedures as known to those of skill in the art. CellSave tubes are disclosed in US Patent Numbers 5,466,574; 5,5 12,332; 5,597,53 1; 5,698,271 ; 5,985,153; 5,993,665; 6,120,856; 6,136,1 82; 6,365,362; 6,55 1,843; 6,620,627; 6,623,982; 6,645,73 1; 6,660,159; 6,790,366; 6,861,259; 6,890,426; 7,01 1,794; 7,282,350; 7,332,288; 5,849,5 17 and 5,459,073, each of which is incorporated by reference in its entirety herein.

[00127] The PAXgene Blood DNA Tube (PAXgene tube) is a plastic, evacuated tube for the collection of whole blood for the isolation of nucleic acids. The tubes can be used for blood collection, transport and storage of whole blood specimens and isolation of nucleic acids contained therein, e.g., DNA or RNA. Blood is collected under a standard phlebotomy protocol into an evacuated tube that contains an additive. The collection and processing can be performed as described in a protocol provided by the manufacturer. PAXgene tubes are disclosed in US Patent Nos. 5,906,744; 4,741,446; 4,991, 104, each of which is incorporated by reference in its entirety herein.

[00128] The RNAlater RNA Stabilization Reagent (RNAlater) is used for immediate stabilization of RNA in tissues. RNA can be unstable in harvested samples. The aqueous RNAlater reagent permeates tissues and other

PREOMICS EXHIBIT 1002
Page 962 of 1861

biological samples, thereby stabilizing and protecting the RNA contained therein. Such protection helps ensure that downstream analyses reflect the expression profile of the RNA in the tissue or other sample. The samples are submerged in an appropriate volume of RNAlater reagent immediately after harvesting. The collection and processing can be performed as described in a protocol provided by the manufacturer. According to the manufacturer, the reagent preserves RNA for up to 1 day at 37°C, 7 days at 18-25°C, or 4 weeks at 2-8°C, allowing processing, transportation, storage, and shipping of samples without liquid nitrogen or dry ice. The samples can also be placed at -20°C or -80°C, e.g., for archival storage. The preserved samples can be used to analyze any type of RNA, including without limitation total RNA, mRNA, and microRNA. RNAlater can also be useful for collecting samples for DNA, RNA and protein analysis. RNAlater is disclosed in US Patent Nos. 5,346,994, each of which is incorporated by reference in its entirety herein.

[00129] Unless otherwise specified, the biological sample of the invention is understood to comprise a sample containing a separated, depleted, enriched, isolated, or otherwise processed derivative of another biological sample. As a non-limiting example, a component of a patient sample or a cell culture can be isolated from the patient sample or the cell culture and resuspended in a buffer for further analysis. One of skill will appreciate that the derivative component suspended in the buffer is a biological sample that can be assessed according to the methods of the invention. The component can be any useful biological entity as disclosed herein or known in the art, including without limitation circulating biomarkers, vesicles, proteins, nucleic acids, lipids or carbohydrates. The biological sample can be the biological entity, including without limitation circulating biomarkers, vesicles, proteins, nucleic acids, lipids or carbohydrates.

Vesicles

[00130] Methods of the invention can include assessing one or more vesicles, including assessing vesicle populations. A vesicle, as used herein, is a membrane vesicle that is shed from cells. Vesicles or membrane vesicles include without limitation: circulating microvesicles (cMVs), microvesicle, exosome, nanovesicle, dexosome, bleb, blebby, prostasome, microparticle, intralumenal vesicle, membrane fragment, intralumenal endosomal vesicle, endosomal-like vesicle, exocytosis vehicle, endosome vesicle, endosomal vesicle, apoptotic body, multivesicular body, secretory vesicle, phospholipid vesicle, liposomal vesicle, argosome, texasome, secresome, tolerosome, melanosome, oncosome, or exocytosed vehicle. Furthermore, although vesicles may be produced by different cellular processes, the methods of the invention are not limited to or reliant on any one mechanism, insofar as such vesicles are present in a biological sample and are capable of being characterized by the methods disclosed herein. Unless otherwise specified, methods that make use of a species of vesicle can be applied to other types of vesicles. Vesicles comprise spherical structures with a lipid bilayer similar to cell membranes which surrounds an inner compartment which can contain soluble components, sometimes referred to as the payload. In some embodiments, the methods of the invention make use of exosomes, which are small secreted vesicles of about 40-100 nm in diameter. For a review of membrane vesicles, including types and characterizations, see *Thery et al, Nat Rev Immunol. 2009 Aug;9(8):581-93.* Some properties of different types of vesicles include those in **Table 2**:

Table 2: Vesicle Properties

Feature	Exosomes	Microvesicles	Ectosomes	Membrane particles	Exosome-like vesicles	Apoptotic vesicles
Size	50-100 nm	100-1,000 nm	50-200 nm	50-80 nm	20-50 nm	50-500 nm
Density in	1.13-1.19 g/ml			1.04-1.07	1.1 g/ml	1.16-1.28

PREOMICS EXHIBIT 1002
Page 963 of 1861

sucrose				g/ml		g/ml
EM appearance	Cup shape	Irregular shape, electron dense	Bilamellar round structures	Round	Irregular shape	Heterogeneous
Sedimentation	100,000 g	10,000 g	160,000-200,000 g	100,000-200,000 g	175,000 g	1,200 g, 10,000 g, 100,000 g
Lipid composition	Enriched in cholesterol, sphingomyelin and ceramide; contains lipid rafts; expose PPS	Expose PPS	Enriched in cholesterol and diacylglycerol; expose PPS		No lipid rafts	
Major protein markers	Tetraspanins (e.g., CD63, CD9), Alix, TSG101	Integrins, selectins and CD40 ligand	CR1 and proteolytic enzymes; no CD63	CD133; no CD63	TNFRI	Histones
Intracellular origin	Internal compartments (endosomes)	Plasma membrane	Plasma membrane	Plasma membrane		

Abbreviations: phosphatidylserine (PPS); electron microscopy (EM)

[00131] Vesicles include shed membrane bound particles, or "microparticles," that are derived from either the plasma membrane or an internal membrane. Vesicles can be released into the extracellular environment from cells. Cells releasing vesicles include without limitation cells that originate from, or are derived from, the ectoderm, endoderm, or mesoderm. The cells may have undergone genetic, environmental, and/or any other variations or alterations. For example, the cell can be tumor cells. A vesicle can reflect any changes in the source cell, and thereby reflect changes in the originating cells, e.g., cells having various genetic mutations. In one mechanism, a vesicle is generated intracellularly when a segment of the cell membrane spontaneously invaginates and is ultimately exocytosed (see for example, *Keller et al, Immunol. Lett. 107 (2): 102-8 (2006)).* Vesicles also include cell-derived structures bounded by a lipid bilayer membrane arising from both herniated evagination (blebbing) separation and sealing of portions of the plasma membrane or from the export of any intracellular membrane-bounded vesicular structure containing various membrane-associated proteins of tumor origin, including surface-bound molecules derived from the host circulation that bind selectively to the tumor-derived proteins together with molecules contained in the vesicle lumen, including but not limited to tumor-derived microRNAs or intracellular proteins. Blebs and blebbing are further described in *Charras et al, Nature Reviews Molecular and Cell Biology, Vol. 9, No. 11, p. 730-736 (2008).* A vesicle shed into circulation or bodily fluids from tumor cells may be referred to as a "circulating tumor-derived vesicle." When such vesicle is an exosome, it may be referred to as a circulating-tumor derived exosome (CTE). In some instances, a vesicle can be derived from a specific cell of origin. CTE, as with a cell-of-origin specific vesicle, typically have one or more unique biomarkers that permit isolation of the CTE or cell-of-origin specific vesicle, e.g., from a bodily fluid and sometimes in a specific manner. For example, a cell or tissue specific markers are used to identify the cell of origin. Examples of such cell or tissue specific markers are disclosed herein and can further be accessed in the Tissue-specific Gene Expression and Regulation (TiGER) Database, available at bioinfo.wilmer.jhu.edu/tiger/; Liu et al. (2008) TiGER: a database for tissue-specific gene expression and

PREOMICS EXHIBIT 1002
Page 964 of 1861

regulation. BMC Bioinformatics. 9:271 ; TissueDistributionDBs, available at genome.dkfz-heidelberg.de/menu/tissue_db/index.html.

[00132] A vesicle can have a diameter of greater than about 10 nm, 20 nm, or 30 nm. A vesicle can have a diameter of greater than 40 nm, 50 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1500 nm, 2000 nm or greater than 10,000 nm. A vesicle can have a diameter of about 20-2000 nm, about 20-1500 nm, about 30-1000 nm, about 30-800 nm, about 30-200 nm, or about 30-100 nm. In some embodiments, the vesicle has a diameter of less than 10,000 nm, 2000 nm,1500 nm, 1000 nm, 800 nm, 500 nm, 200 nm, 100 nm, 50 nm, 40 nm, 30 nm, 20 nm or less than 10 nm. As used herein the term "about" in reference to a numerical value means that variations of 10% above or below the numerical value are within the range ascribed to the specified value. Typical sizes for various types of vesicles are shown in **Table 2.** Vesicles can be assessed to measure the diameter of a single vesicle or any number of vesicles. For example, the range of diameters of a vesicle population or an average diameter of a vesicle population can be determined. Vesicle diameter can be assessed using methods known in the art, e.g., imaging technologies such as electron microscopy. In an embodiment, a diameter of one or more vesicles is determined using optical particle detection. See, e.g., U.S. Patent 7,75 1,053, entitled "Optical Detection and Analysis of Particles" and issued July 6, 2010; and U.S. Patent 7,399,600, entitled "Optical Detection and Analysis of Particles" and issued July 15, 2010.

[00133] In some embodiments, vesicles are directly assayed from a biological sample without prior isolation, purification, or concentration from the biological sample. For example, the amount of vesicles in the sample can by itself provide a biosignature that provides a diagnostic, prognostic or theranostic determination. Alternatively, the vesicle in the sample may be isolated, captured, purified, or concentrated from a sample prior to analysis. As noted, isolation, capture or purification as used herein comprises partial isolation, partial capture or partial purification apart from other components in the sample. Vesicle isolation can be performed using various techniques as described herein, e.g., chromatography, filtration, centrifugation, flow cytometry, affinity capture (e.g., to a planar surface or bead), and/or using microfluidics.

[00134] Vesicles such as exosomes can be assessed to provide a phenotypic characterization by comparing vesicle characteristics to a reference. In some embodiments, surface antigens on a vesicle are assessed. The surface antigens can provide an indication of the anatomical origin and/or cellular of the vesicles and other phenotypic information, e.g., tumor status. For example, wherein vesicles found in a patient sample, e.g., a bodily fluid such as blood, serum or plasma, are assessed for surface antigens indicative of colorectal origin and the presence of cancer. The surface antigens may comprise any informative biological entity that can be detected on the vesicle membrane surface, including without limitation surface proteins, lipids, carbohydrates, and other membrane components. For example, positive detection of colon derived vesicles expressing tumor antigens can indicate that the patient has colorectal cancer. As such, methods of the invention can be used to characterize any disease or condition associated with an anatomical or cellular origin, by assessing, for example, disease-specific and cell-specific biomarkers of one or more vesicles obtained from a subject.

[00135] In another embodiment, one or more vesicle payloads are assessed to provide a phenotypic characterization. The payload with a vesicle comprises any informative biological entity that can be detected as encapsulated within the vesicle, including without limitation proteins and nucleic acids, e.g., genomic or cDNA, mRNA, or functional fragments thereof, as well as microRNAs (miRs). In addition, methods of the invention are directed to detecting vesicle surface antigens (in addition or exclusive to vesicle payload) to provide a

PREOMICS EXHIBIT 1002
Page 965 of 1861

phenotypic characterization. For example, vesicles can be characterized by using binding agents (e.g., antibodies or aptamers) that are specific to vesicle surface antigens, and the bound vesicles can be further assessed to identify one or more payload components disclosed therein. As described herein, the levels of vesicles with surface antigens of interest or with payload of interest can be compared to a reference to characterize a phenotype. For example, overexpression in a sample of cancer-related surface antigens or vesicle payload, e.g., a tumor associated mRNA or microRNA, as compared to a reference, can indicate the presence of cancer in the sample. The biomarkers assessed can be present or absent, increased or reduced based on the selection of the desired target sample and comparison of the target sample to the desired reference sample. Non-limiting examples of target samples include: disease; treated/not-treated; different time points, such as a in a longitudinal study; and non-limiting examples of reference sample: non-disease; normal; different time points; and sensitive or resistant to candidate treatment(s).

MicroRNA

[00136] Various biomarker molecules can be assessed in biological samples or vesicles obtained from such biological samples. MicroRNAs comprise one class biomarkers assessed via methods of the invention. MicroRNAs, also referred to herein as miRNAs or miRs, are short RNA strands approximately 21-23 nucleotides in length. MiRNAs are encoded by genes that are transcribed from DNA but are not translated into protein and thus comprise non-coding RNA. The miRs are processed from primary transcripts known as pri-miRNA to short stem-loop structures called pre-miRNA and finally to the resulting single strand miRNA. The pre-miRNA typically forms a structure that folds back on itself in self-complementary regions. These structures are then processed by the nuclease Dicer in animals or DCL1 in plants. Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules and can function to regulate translation of proteins. Identified sequences of miRNA can be accessed at publicly available databases, such as www.microRNA.org, www.mirbase.org, or www.mirz.unibas.ch/cgi/miRNA.cgi.

[00137] miRNAs are generally assigned a number according to the naming convention " mir-[number]." The number of a miRNA is assigned according to its order of discovery relative to previously identified miRNA species. For example, if the last published miRNA was mir-121, the next discovered miRNA will be named mir-122, etc. When a miRNA is discovered that is homologous to a known miRNA from a different organism, the name can be given an optional organism identifier, of the form [organism identifier]- mir-[number]. Identifiers include hsa for Homo sapiens and mmu for Mus Musculus. For example, a human homolog to mir-121 might be referred to as hsa-mir-121 whereas the mouse homolog can be referred to as mmu-mir-121 and the rat homolog can be referred to as rno-mir-121, etc.

[00138] Mature microRNA is commonly designated with the prefix "miR" whereas the gene or precursor miRNA is designated with the prefix "mir." For example, mir-121 is a precursor for miR-121. When differing miRNA genes or precursors are processed into identical mature miRNAs, the genes/precursors can be delineated by a numbered suffix. For example, mir-121-1 and mir-121-2 can refer to distinct genes or precursors that are processed into miR-121. Lettered suffixes are used to indicate closely related mature sequences. For example, mir-121a and mir-121b can be processed to closely related miRNAs miR-121a and miR-121b, respectively. In the context of the invention, any microRNA (miRNA or miR) designated herein with the prefix mir-* or miR-* is understood to encompass both the precursor and/or mature species, unless otherwise explicitly stated otherwise.

PREOMICS EXHIBIT 1002
Page 966 of 1861

[00139] Sometimes it is observed that two mature miRNA sequences originate from the same precursor. When one of the sequences is more abundant that the other, a "*" suffix can be used to designate the less common variant. For example, miR-121 would be the predominant product whereas miR-121 * is the less common variant found on the opposite arm of the precursor. If the predominant variant is not identified, the miRs can be distinguished by the suffix "5p" for the variant from the 5' arm of the precursor and the suffix "3p" for the variant from the 3' arm. For example, miR-121-5p originates from the 5' arm of the precursor whereas miR-121-3p originates from the 3' arm. Less commonly, the 5p and 3p variants are referred to as the sense ("s") and anti-sense ("as") forms, respectively. For example, miR-121-5p may be referred to as miR-121-s whereas miR-121-3p may be referred to as miR-121-as.

[00140] The above naming conventions have evolved over time and are general guidelines rather than absolute rules. For example, the let- and lin- families of miRNAs continue to be referred to by these monikers. The mir/miR convention for precursor/mature forms is also a guideline and context should be taken into account to determine which form is referred to. Further details of miR naming can be found at www.mirbase.org or Ambros et al., A uniform system for microRNA annotation, RNA **9**:277-279 (2003).

[00141] Plant miRNAs follow a different naming convention as described in Meyers et al., Plant Cell. 2008 20(12):3 186-3 190.

[00142] A number of miRNAs are involved in gene regulation, and miRNAs are part of a growing class of non-coding RNAs that is now recognized as a major tier of gene control. In some cases, miRNAs can interrupt translation by binding to regulatory sites embedded in the 3'-UTRs of their target mRNAs, leading to the repression of translation. Target recognition involves complementary base pairing of the target site with the miRNA's seed region (positions 2-8 at the miRNA's 5' end), although the exact extent of seed complementarity is not precisely determined and can be modified by 3' pairing. In other cases, miRNAs function like small interfering RNAs (siRNA) and bind to perfectly complementary mRNA sequences to destroy the target transcript.

[00143] Characterization of a number of miRNAs indicates that they influence a variety of processes, including early development, cell proliferation and cell death, apoptosis and fat metabolism. For example, some miRNAs, such as lin-4, let-7, mir-14, mir-23, and bantam, have been shown to play critical roles in cell differentiation and tissue development. Others are believed to have similarly important roles because of their differential spatial and temporal expression patterns.

[00144] The miRNA database available at miRBase (www.mirbase.org) comprises a searchable database of published miRNA sequences and annotation. Further information about miRBase can be found in the following articles, each of which is incorporated by reference in its entirety herein: Griffiths-Jones et al., miRBase: tools for microRNA genomics. NAR 2008 36(Database Issue):D154-D158; Griffiths-Jones et al., miRBase: microRNA sequences, targets and gene nomenclature. NAR 2006 34(Database Issue):D140-D144; and Griffiths-Jones, S. The microRNA Registry. NAR 2004 32(Database Issue):D109-Dl 11. Representative miRNAs contained in Release 16 of miRBase, made available September 2010.

[00145] As described herein, microRNAs are known to be involved in cancer and other diseases and can be assessed in order to characterize a phenotype in a sample. See, e.g., Ferracin et al., Micromarkers: miRNAs in cancer diagnosis and prognosis, Exp Rev Mol Diag, Apr 2010, Vol. 10, No. 3, Pages 297-308; Fabbri, miRNAs as molecular biomarkers of cancer, Exp Rev Mol Diag, May 2010, Vol. 10, No. 4, Pages 435-444. Techniques

PREOMICS EXHIBIT 1002
Page 967 of 1861

to isolate and characterize vesicles and miRs are known to those of skill in the art. In addition to the methodology presented herein, additional methods can be found in U.S. Patent No. 7,888,035, entitled "METHODS FOR ASSESSING RNA PATTERNS" and issued February 15, 201 1; and International Patent Application Nos. PCT/US20 10/05 8461, entitled "METHODS AND SYSTEMS FOR ISOLATING, STORING, AND ANALYZING VESICLES" and filed November 30, 2010; and PCT/US201 1/021 160, entitled "DETECTION OF GASTROINTESTINAL DISORDERS" and filed January 13, 201 1; each of which applications are incorporated by reference herein in their entirety.

Circulating Biomarkers

[00146] Circulating biomarkers include biomarkers that are detectable in body fluids, such as blood, plasma, serum. Examples of circulating cancer biomarkers include cardiac troponin T (cTnT), prostate specific antigen (PSA) for prostate cancer and CA125 for ovarian cancer. Circulating biomarkers according to the invention include any appropriate biomarker that can be detected in bodily fluid, including without limitation protein, nucleic acids, e.g., DNA, mRNA and microRNA, lipids, carbohydrates and metabolites. Circulating biomarkers can include biomarkers that are not associated with cells, such as biomarkers that are membrane associated, embedded in membrane fragments, part of a biological complex, or free in solution. In one embodiment, circulating biomarkers are biomarkers that are associated with one or more vesicles present in the biological fluid of a subject.

[00147] Circulating biomarkers have been identified for use in characterization of various phenotypes. See, e.g., Ahmed N, et al., Proteomic -based identification of haptoglobin- 1 precursor as a novel circulating biomarker of ovarian cancer. Br. J. Cancer 2004; Mathelin et al., Circulating proteinic biomarkers and breast cancer, Gynecol Obstet Fertil. 2006 Jul-Aug;34(7-8):638-46. Epub 2006 Jul 28; Ye et al., Recent technical strategies to identify diagnostic biomarkers for ovarian cancer. Expert Rev Proteomics. 2007 Feb;4(l): 121-3 1; Carney, Circulating oncoproteins HER2/neu, EGFR and CALX (MN) as novel cancer biomarkers. Expert Rev Mol Diagn. 2007 May;7(3):309-19; Gagnon, Discovery and application of protein biomarkers for ovarian cancer, Curr Opin Obstet Gynecol. 2008 Feb;20(l):9-13; Pasterkamp et al., Immune regulatory cells: circulating biomarker factories in cardiovascular disease. Clin Sci (Lond). 2008 Aug; 115(4): 129-3 1; Fabbri, miRNAs as molecular biomarkers of cancer, Exp Rev Mol Diag, May 2010, Vol. 10, No. 4, Pages 435-444; PCT Patent Publication WO/2007/088537; U.S. Patents 7,745,150 and 7,655,479; U.S. Patent Publications 201 10008808, 20100330683, 20100248290, 20100222230, 20100203566, 20100173788, 20090291932, 20090239246, 20090226937, 200901 11121, 20090004687, 20080261258, 20080213907, 20060003465, 20050124071, and 20040096915, each of which publication is incorporated herein by reference in its entirety.

Vesicle Enrichment

[00148] A vesicle or a population of vesicles may be isolated, purified, concentrated or otherwise enriched prior to and/or during analysis. Unless otherwise specified, the terms "purified," "isolated," or similar as used herein in reference to vesicles or biomarker components are intended to include partial or complete purification or isolation of such components from a cell or organism. Analysis of a vesicle can include quantitiating the amount one or more vesicle populations of a biological sample. For example, a heterogeneous population of vesicles can be quantitated, or a homogeneous population of vesicles, such as a population of vesicles with a particular biomarker profile, a particular biosignature, or derived from a particular cell type can be isolated from a heterogeneous population of vesicles and quantitated. Analysis of a vesicle can also include detecting,

PREOMICS EXHIBIT 1002
Page 968 of 1861

quantitatively or qualitatively, one or more particular biomarker profile or biosignature of a vesicle, as described herein.

[00149] A vesicle can be stored and archived, such as in a bio-fluid bank and retrieved for analysis as necessary. A vesicle may also be isolated from a biological sample that has been previously harvested and stored from a living or deceased subject. In addition, a vesicle may be isolated from a biological sample which has been collected as described in *King et al, Breast Cancer Res 7(5): 198-204 (2005).* A vesicle can be isolated from an archived or stored sample. Alternatively, a vesicle may be isolated from a biological sample and analyzed without storing or archiving of the sample. Furthermore, a third party may obtain or store the biological sample, or obtain or store the vesicle for analysis.

[00150] An enriched population of vesicles can be obtained from a biological sample. For example, vesicles may be concentrated or isolated from a biological sample using size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof.

[00151] Size exclusion chromatography, such as gel permeation columns, centrifugation or density gradient centrifugation, and filtration methods can be used. For example, a vesicle can be isolated by differential centrifugation, anion exchange and/or gel permeation chromatography (for example, as described in US Patent Nos. 6,899,863 and 6,812,023), sucrose density gradients, organelle electrophoresis (for example, as described in U.S. Patent No. 7,198,923), magnetic activated cell sorting (MACS), or with a nanomembrane ultrafiltration concentrator. Various combinations of isolation or concentration methods can be used.

[00152] Highly abundant proteins, such as albumin and immunoglobulin, may hinder isolation of vesicles from a biological sample. For example, a vesicle can be isolated from a biological sample using a system that uses multiple antibodies that are specific to the most abundant proteins found in a biological sample, such as blood. Such a system can remove up to several proteins at once, thus unveiling the lower abundance species such as cell-of-origin specific vesicles.

[00153] This type of system can be used for isolation of vesicles from biological samples such as blood, cerebrospinal fluid or urine. The isolation of vesicles from a biological sample may also be enhanced by high abundant protein removal methods as described in *Chromy et al. J Proteome Res 2004; 3:1120-1127.* In another embodiment, the isolation of vesicles from a biological sample may also be enhanced by removing serum proteins using glycopeptide capture as described in *Zhang et al, Mol Cell Proteomics 2005;4:144-155.* In addition, vesicles from a biological sample such as urine may be isolated by differential centrifugation followed by contact with antibodies directed to cytoplasmic or anti-cytoplasmic epitopes as described in *Pisitkun et al, Proc Natl Acad Sci USA, 2004;101:13368-13373.*

[00154] Isolation or enrichment of a vesicle from a biological sample can also be enhanced by use of sonication (for example, by applying ultrasound), detergents, other membrane-activating agents, or any combination thereof. For example, ultrasonic energy can be applied to a potential tumor site, and without being bound by theory, release of vesicles from a tissue can be increased, allowing an enriched population of vesicles that can be analyzed or assessed from a biological sample using one or more methods disclosed herein.

Sample Handling

[00155] With methods of detecting circulating biomarkers as described here, e.g., antibody affinity isolation, the consistency of the results can be optimized as necessary using various concentration or isolation procedures.

PREOMICS EXHIBIT 1002
Page 969 of 1861

Such steps can include agitation such as shaking or vortexing, different isolation techniques such as polymer based isolation, e.g., with PEG, and concentration to different levels during filtration or other steps. It will be understood by those in the art that such treatments can be applied at various stages of testing the vesicle containing sample. In one embodiment, the sample itself, e.g., a bodily fluid such as plasma or serum, is vortexed. In some embodiments, the sample is vortexed after one or more sample treatment step, e.g., vesicle isolation, has occurred. Agitation can occur at some or all appropriate sample treatment steps as desired. Additives can be introduced at the various steps to improve the process, e.g., to control aggregation or degradation of the biomarkers of interest.

[00156] The results can also be optimized as desireable by treating the sample with various agents. Such agents include additives to control aggregation and/or additives to adjust pH or ionic strength. Additives that control aggregation include blocking agents such as bovine serum albumin (BSA), milk or StabilGuard® (a BSA-free blocking agent; Product code SG02, Surmodics, Eden Prairie, MN), chaotropic agents such as guanidium hydro chloride, and detergents or surfactants. Useful ionic detergents include sodium dodecyl sulfate (SDS, sodium lauryl sulfate (SLS)), sodium laureth sulfate (SLS, sodium lauryl ether sulfate (SLES)), ammonium lauryl sulfate (ALS), cetrimonium bromide, cetrimonium chloride, cetrimonium stearate, and the like. Useful non-ionic (zwitterionic) detergents include polyoxyethylene glycols, polysorbate 20 (also known as Tween 20), other polysorbates (e.g., 40, 60, 65, 80, etc), Triton-X (e.g., X100, XI 14), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), CHAPSO, deoxycholic acid, sodium deoxycholate, NP-40, glycosides, octyl-thio-glucosides, maltosides, and the like. In some embodiments, Pluronic F-68, a surfactant shown to reduce platelet aggregation, is used to treat samples containing vesicles during isolation and/or detection. F68 can be used from a 0.1% to 10% concentration, e.g., a 1%, 2.5% or 5% concentration. The pH and/or ionic strength of the solution can be adjusted with various acids, bases, buffers or salts, including without limitation sodium chloride (NaCl), phosphate-buffered saline (PBS), tris-buffered saline (TBS), sodium phosphate, potassium chloride, potassium phosphate, sodium citrate and saline-sodium citrate (SSC) buffer. In some embodiments, NaCl is added at a concentration of 0.1% to 10%, e.g., 1%, 2.5% or 5% final concentration. In some embodiments, Tween 20 is added to 0.005 to 2% concentration, e.g., 0.05%, 0.25% or 0.5 % final concentration. Blocking agents for use with the invention comprise inert proteins, e.g., milk proteins, non-fat dry milk protein, albumin, BSA, casein, or serum such as newborn calf serum (NBCS), goat serum, rabbit serum or salmon serum. The proteins can be added at a 0.1% to 10% concentration, e.g., 1%, 2%, 3%, 3.5%, 4%, 5%, 6%, 7%, 8%, 9% or 10% concentration. In some embodiments, BSA is added to 0.1% to 10% concentration, e.g., 1%, 2%, 3%, 3.5%, 4%, 5%, 6%, 7%, 8%, 9% or 10% concentration. In an embodiment, the sample is treated according to the methodology presented in U.S. Patent Application 11/632946, filed July 13, 2005, which application is incorporated herein by reference in its entirety. Commercially available blockers may be used, such as SuperBlock, StartingBlock, Protein-Free from Pierce (a division of Thermo Fisher Scientific, Rockford, IL). In some embodiments, SSC/detergent (e.g., 20X SSC with 0.5% Tween 20 or 0.1% Triton-X 100) is added to 0.1% to 10% concentration, e.g., at 1.0% or 5.0% concentration.

[00157] The methods of detecting vesicles and other circulating biomarkers can be optimized as desired with various combinations of protocols and treatments as described herein. A detection protocol can be optimized by various combinations of agitation, isolation methods, and additives. In some embodiments, the patient sample is vortexed before and after isolation steps, and the sample is treated with blocking agents including BSA and/or

PREOMICS EXHIBIT 1002
Page 970 of 1861

F68. Such treatments may reduce the formation of large aggregates or protein or other biological debris and thus provide a more consistent detection reading.

Filtration and Ultrafiltration

[00158] A vesicle can be isolated from a biological sample by filtering a biological sample from a subject through a filtration module and collecting from the filtration module a retentate comprising the vesicle, thereby isolating the vesicle from the biological sample. The method can comprise filtering a biological sample from a subject through a filtration module comprising a filter; and collecting from the filtration module a retentate comprising the vesicle, thereby isolating the vesicle from the biological sample. In one embodiment, the filter retains molecules greater than about 100 kiloDaltons.

[00159] The method can further comprise determining a biosignature of the vesicle. The method can also further comprise applying the retentate to a plurality of substrates, wherein each substrate is coupled to one or more capture agents, and each subset of the plurality of substrates comprises a different capture agent or combination of capture agents than another subset of the plurality of substrates.

[00160] Also provided herein is a method of determining a biosignature of a vesicle in a sample comprising: filtering a biological sample from a subject with a disorder through a filtration module, collecting from the filtration module a retentate comprising one or more vesicles, and determining a biosignature of the one or more vesicles. In one embodiment, the filtration module comprises a filter that retains molecules greater than about 100 or 150 kiloDaltons.

[00161] The method disclosed herein can further comprise characterizing a phenotype in a subject by filtering a biological sample from a subject through a filtration module, collecting from the filtration module a retentate comprising one or more vesicles; detecting a biosignature of the one or more vesicles; and characterizing a phenotype in the subject based on the biosignature, wherein characterizing is with at least 70% sensitivity. In some embodiments, characterizing comprises determining an amount of one or more vesicle having the biosignature. Furthermore, the characterizing can be from about 80% to 100% sensitivity.

[00162] Also provided herein is a method for multiplex analysis of a plurality of vesicles. In some embodiments, the method comprises filtering a biological sample from a subject through a filtration module; collecting from the filtration module a retentate comprising the plurality of vesicles, applying the plurality of vesicles to a plurality of capture agents, wherein the plurality of capture agents is coupled to a plurality of substrates, and each subset of the plurality of substrates is differentially labeled from another subset of the plurality of substrates; capturing at least a subset of the plurality of vesicles; and determining a biosignature for at least a subset of the captured vesicles. In one embodiment, each substrate is coupled to one or more capture agents, and each subset of the plurality of substrates comprises a different capture agent or combination of capture agents as compared to another subset of the plurality of substrates. In some embodiments, at least a subset of the plurality of substrates is intrinsically labeled, such as comprising one or more labels. The substrate can be a particle or bead, or any combination thereof. In some embodiments, the filter retains molecules greater than 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, or 500 kiloDaltons. In one embodiment, the filtration module comprises a filter that retains molecules greater than about 100 or 150 kiloDaltons. In one embodiment, the filtration module comprises a filter that retains molecules greater than about 9, 20, 100 or 150 kiloDaltons.

PREOMICS EXHIBIT 1002
Page 971 of 1861

[00163] In some embodiments, the method for multiplex analysis of a plurality of vesicles comprises filtering a biological sample from a subject through a filtration module, wherein the filtration module comprises a filter that retains molecules greater than about 100 kiloDaltons; collecting from the filtration module a retentate comprising the plurality of vesicles; applying the plurality of vesicles to a plurality of capture agents, wherein the plurality of capture agents is coupled to a microarray; capturing at least a subset of the plurality of vesicles on the microarray; and determining a biosignature for at least a subset of the captured vesicles. In some embodiments, the filter retains molecules greater than 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, or 500 kiloDaltons. In one embodiment, the filtration module comprises a filter that retains molecules greater than about 100 or 150 kiloDaltons. In one embodiment, the filtration module comprises a filter that retains molecules greater than about 9, 20, 100 or 150 kiloDaltons.

[00164] The biological sample can be clarified prior to isolation by filtration. Clarification comprises selective removal of cellular debris and other undesirable materials. For example, cellular debris and other components that may interfere with detection of the circulating biomarkers can be removed. The clarification can be by low-speed centrifugation, such as at about 5,000x g, 4,000x g, 3,000x g, 2,000x g, 1,000x g, or less. The supernatant, or clarified biological sample, containing the vesicle can then be collected and filtered to isolate the vesicle from the clarified biological sample. In some embodiments, the biological sample is not clarified prior to isolation of a vesicle by filtration.

[00165] In some embodiments, isolation of a vesicle from a sample does not use high-speed centrifugation, such as ultracentrifugation. For example, isolation may not require the use of centrifugal speeds, such as about 100,000x g or more. In some embodiments, isolation of a vesicle from a sample uses speeds of less than 50,000 x g, 40,000 x g, 30,000 x g, 20,000 x g, 15,000 x g, 12,000 x g, or 10,000 x g.

[00166] Any number of applicable filter configurations can be used to filter a sample of interest. In some embodiments, the filtration module used to isolate the circulating biomarkers from the biological sample is a fiber-based filtration cartridge. For example, the fiber can be a hollow polymeric fiber, such as a polypropylene hollow fiber. A biological sample can be introduced into the filtration module by pumping the sample fluid, such as a biological fluid as disclosed herein, into the module with a pump device, such as a peristaltic pump. The pump flow rate can vary, such as at about 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, or 10 mL/minute. The flow rate can be adjusted given the configuration, e.g., size and throughput, of the filtration module.

[00167] The filtration module can be a membrane filtration module. For example, the membrane filtration module can comprise a filter disc membrane, such as a hydrophilic polyvinylidene difluoride (PVDF) filter disc membrane housed in a stirred cell apparatus (e.g., comprising a magnetic stirrer). In some embodiments, the sample moves through the filter as a result of a pressure gradient established on either side of the filter membrane.

[00168] The filter can comprise a material having low hydrophobic absorptivity and/or high hydrophilic properties. For example, the filter can have an average pore size for vesicle retention and permeation of most proteins as well as a surface that is hydrophilic, thereby limiting protein adsorption. For example, the filter can comprise a material selected from the group consisting of polypropylene, PVDF, polyethylene, polyfluoroethylene, cellulose, secondary cellulose acetate, polyvinylalcohol, and ethylenevinyl alcohol

PREOMICS EXHIBIT 1002
Page 972 of 1861

(EVAL®, Kuraray Co., Okayama, Japan). Additional materials that can be used in a filter include, but are not limited to, polysulfone and polyethersulfone.

[00169] The filtration module can have a filter that retains molecules greater than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, or 500 kiloDaltons (kDa), such as a filter that has a MWCO (molecular weight cut off) of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, or 500 kDa. Ultrafiltration membranes with a range of MWCO of 9 kDa, 20 kDa and/or 150 kDa can be used. In some embodiments, the filter within the filtration module has an average pore diameter of about 0.01 μπι to about 0.15 μπι, and in some embodiments from about 0.05 μπι to about 0.12 μπι. In some embodiments, the filter has an average pore diameter of about 0.06 μπι, 0.07 μιη, 0.08 μπι, 0.09 μιη, 0.1 μπι, 0.1 1 μπι or 0.2 μτη.

[00170] The filtration module can be a commerically available column, such as a column typically used for concentrating proteins or for isolating proteins (e.g., ultrafiltration). Examples include, but are not limited to, columns from Millpore (Billerica, MA), such as Amicon® centrifugal filters, or from Pierce® (Rockford, IL), such as Pierce Concentrator filter devices. Useful columns from Pierce include disposable ultrafiltration centrifugal devices with a MWCO of 9 kDa, 20 kDa and/or 150 kDa. These concentrators consist of a high-performance regenerated cellulose membrane welded to a conical device. The filters can be as described in U.S. Patents 6,269,957 or 6,357,601, both of which applications are incorporated by reference in their entirety herein.

[00171] The retentate comprising the isolated vesicle can be collected from the filtration module. The retentate can be collected by flushing the retentate from the filter. Selection of a filter composition having hydrophilic surface properties, thereby limiting protein adsorption, can be used, without being bound by theory, for easier collection of the retentate and minimize use of harsh or time-consuming collection techniques.

[00172] The collected retentate can then be used subsequent analysis, such as assessing a biosignature of one or more vesicles in the retentate, as further described herein. The analysis can be directly performed on the collected retentate. Alternatively, the collected retentate can be further concentrated or purified, prior to analysis of one or more vesicles. For example, the retentate can be further concentrated or vesicles further isolated from the retentate using size exclusion chromatography, density gradient centrifugation, differential centrifugation, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof, such as described herein. In some embodiments, the retentate can undergo another step of filtration. Alternatively, prior to isolation of a vesicle using a filter, the vesicle is concentrated or isolated using size exclusion chromatography, density gradient centrifugation, differential centrifugation, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof

[00173] Combinations of filters can be used for concentrating and isolating biomarkers. For example, the biological sample may first be filtered through a filter having a porosity or pore size of between about 0.01 μιη to about 2 μιη, about 0.05 μιη to about 1.5 μιη, and then the sample is filtered. For example, prior to filtering a biological sample through a filtration module with a filter that retains molecules greater than about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, or 500 kiloDaltons (kDa), such as a filter that has a MWCO (molecular weight cut off) of about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, or 500, the biological sample may first be filtered through a filter having a porosity or pore size of between about 0.01 μιη to about 2 μιη, about 0.05 μιη to about 1.5 μιη, In some

PREOMICS EXHIBIT 1002
Page 973 of 1861

embodiments, the filter has a pore size of about 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 μπι. The filter may be a syringe filter. Thus, in one embodiment, the method comprises filtering the biological sample through a filter, such as a syringe filter, wherein the syringe filter has a porosity of greater than about 1 μπι, prior to filtering the sample through a filtration module comprising a filter that retains molecules greater than about 100 or 150 kiloDaltons. In an embodiment, the filter is 1.2 μM filter and the filtration is followed by passage of the sample through a 7 ml or 20 ml concentrator column with a 150 kDa cutoff.

[00174] The filtration module can be a component of a microfluidic device. Microfluidic devices, which may also be referred to as "lab-on-a-chip" systems, biomedical micro-electro-mechanical systems (bioMEMs), or multicomponent integrated systems, can be used for isolating, and analyzing, vesicles. Such systems miniaturize and compartmentalize processes that allow for binding of vesicles, detection of biomarkers, and other processes, such as further described herein.

[00175] The filtration module and assessment can be as described in Grant, R., et al., A filtration-based protocol to isolate human Plasma Membrane-derived Vesicles and exosomes from blood plasma, *J Immunol Methods* (**201 1**) **371**:143-5 1 (Epub 201 1 Jun 30), which reference is incorporated herein by reference in its entirety.

[00176] A microfluidic device can also be used for isolation of a vesicle by comprising a filtration module. For example, a microfluidic device can use one more channels for isolating a vesicle from a biological sample based on size from a biological sample. A biological sample can be introduced into one or more microfluidic channels, which selectively allows the passage of vesicles. The microfluidic device can further comprise binding agents, or more than one filtration module to select vesicles based on a property of the vesicles, for example, size, shape, deformability, biomarker profile, or biosignature.

Binding Agents

[00177] Binding agents (also referred to as binding reagents) include agents that are capable of binding a target biomarker. A binding agent can be specific for the target biomarker, meaning the agent is capable of binding a target biomarker. The target can be any useful biomarker disclosed herein, such as a biomarker on the vesicle surface. In some embodiments, the target is a single molecule, such as a single protein, so that the binding agent is specific to the single protein. In other embodiments, the target can be a group of molecules, such as a family or proteins having a similar epitope or moiety, so that the binding agent is specific to the family or group of proteins. The group of molecules can also be a class of molecules, such as protein, DNA or RNA. The binding agent can be a capture agent used to capture a vesicle by binding a component or biomarker of a vesicle. In some embodiments, a capture agent comprises an antibody or fragment thereof, or an aptamer, that binds to an antigen on a vesicle. The capture agent can be optionally coupled to a substrate and used to isolate a vesicle, as further described herein.

[00178] A binding agent is an agent that binds to a circulating biomarker, such as a vesicle or a component of a vesicle. The binding agent can be used as a capture agent and/or a detection agent. A capture agent can bind and capture a circulating biomarker, such as by binding a component or biomarker of a vesicle. For example, the capture agent can be a capture antibody or capture antigen that binds to an antigen on a vesicle. A detection agent can bind to a circulating biomarker thereby facilitating detection of the biomarker. For example, a capture agent comprising an antibody or aptamer that is sequestered to a substrate can be used to capture a vesicle in a sample, and a detection agent comprising an antibody or aptamer that carries a label can be used to detect the

PREOMICS EXHIBIT 1002
Page 974 of 1861

captured vesicle via detection of the detection agent's label. In some embodiments, a vesicle is assessed using capture and detection agents that recognize the same vesicle biomarkers. For example, a vesicle population can be captured using a tetraspanin such as by using an anti-CD9 antibody bound to a substrate, and the captured vesicles can be detected using a fluorescently labeled anti-CD9 antibody to label the captured vesicles. In other embodiments, a vesicle is assessed using capture and detection agents that recognize different vesicle biomarkers. For example, a vesicle population can be captured using a cell-specific marker such as by using an anti-PCSA antibody bound to a substrate, and the captured vesicles can be detected using a fluorescently labeled anti-CD9 antibody to label the captured vesicles. Similarly, the vesicle population can be captured using a general vesicle marker such as by using an anti-CD9 antibody bound to a substate, and the captured vesicles can be detected using a fluorescently labeled antibody to a cell-specific or disease specific marker to label the captured vesicles.

[00179] The biomarkers recognized by the binding agent are sometimes referred to herein as an antigen. Unless otherwise specified, antigen as used herein is meant to encompass any entity that is capable of being bound by a binding agent, regardless of the type of binding agent or the immunogenicity of the biomarker. The antigen further encompasses a functional fragment thereof. For example, an antigen can encompass a protein biomarker capable of being bound by a binding agent, including a fragment of the protein that is capable of being bound by a binding agent.

[00180] In one embodiment, a vesicle is captured using a capture agent that binds to a biomarker on a vesicle. The capture agent can be coupled to a substrate and used to isolate a vesicle, as further described herein. In one embodiment, a capture agent is used for affinity capture or isolation of a vesicle present in a substance or sample.

[00181] A binding agent can be used after a vesicle is concentrated or isolated from a biological sample. For example, a vesicle can first be isolated from a biological sample before a vesicle with a specific biosignature is isolated or detected. The vesicle with a specific biosignature can be isolated or detected using a binding agent for the biomarker. A vesicle with the specific biomarker can be isolated or detected from a heterogeneous population of vesicles. Alternatively, a binding agent may be used on a biological sample comprising vesicles without a prior isolation or concentration step. For example, a binding agent is used to isolate or detect a vesicle with a specific biosignature directly from a biological sample.

[00182] A binding agent can be a nucleic acid, protein, or other molecule that can bind to a component of a vesicle. The binding agent can comprise DNA, RNA, monoclonal antibodies, polyclonal antibodies, Fabs, Fab', single chain antibodies, synthetic antibodies, aptamers (DNA/RNA), peptoids, zDNA, peptide nucleic acids (PNAs), locked nucleic acids (LNAs), lectins, synthetic or naturally occurring chemical compounds (including but not limited to drugs, labeling reagents), dendrimers, or a combination thereof. For example, the binding agent can be a capture antibody. In embodiments of the invention, the binding agent comprises a membrane protein labeling agent. See, e.g., the membrane protein labeling agents disclosed in Alroy et al., US. Patent Publication US 2005/0158708. In an embodiment, vesicles are isolated or captured as described herein, and one or more membrane protein labeling agent is used to detect the vesicles.

[00183] In some instances, a single binding agent can be employed to isolate or detect a vesicle. In other instances, a combination of different binding agents may be employed to isolate or detect a vesicle. For example, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 different

PREOMICS EXHIBIT 1002
Page 975 of 1861

binding agents may be used to isolate or detect a vesicle from a biological sample. Furthermore, the one or more different binding agents for a vesicle can form a biosignature of a vesicle, as further described below.

[00184] Different binding agents can also be used for multiplexing. For example, isolation or detection of more than one population of vesicles can be performed by isolating or detecting each vesicle population with a different binding agent. Different binding agents can be bound to different particles, wherein the different particles are labeled. In another embodiment, an array comprising different binding agents can be used for multiplex analysis, wherein the different binding agents are differentially labeled or can be ascertained based on the location of the binding agent on the array. Multiplexing can be accomplished up to the resolution capability of the labels or detection method, such as described below. The binding agents can be used to detect the vesicles, such as for detecting cell-of-origin specific vesicles. A binding agent or multiple binding agents can themselves form a binding agent profile that provides a biosignature for a vesicle. One or more binding agents can be selected from Fig. 2 of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein. For example, if a vesicle population is detected or isolated using two, three, four or more binding agents in a differential detection or isolation of a vesicle from a heterogeneous population of vesicles, the particular binding agent profile for the vesicle population provides a biosignature for the particular vesicle population. The vesicle can be detected using any number of binding agents in a multiplex fashion. Thus, the binding agent can also be used to form a biosignature for a vesicle. The biosignature can be used to characterize a phenotype.

[00185] The binding agent can be a lectin. Lectins are proteins that bind selectively to polysaccharides and glycoproteins and are widely distributed in plants and animals. For example, lectins such as those derived from Galanthus nivalis in the form of Galanthus nivalis agglutinin ("GNA"), Narcissus pseudonarcissus in the form of Narcissus pseudonarcissus agglutinin ("NPA") and the blue green algae Nostoc ellipsosporum called "cyanovirin" *{Boyd et al. Antimicrob Agents Chemother 41(7): 1521 1530, 1997; Hammar et al. Ann N YAcad Sci 724: 166 169, 1994; Kaku et al Arch Biochem Biophys 279(2): 298 304, 1990)* can be used to isolate a vesicle. These lectins can bind to glycoproteins having a high mannose content *(Chervenak et al. Biochemistry 34(16): 5685 5695, 1995).* High mannose glycoprotein refers to glycoproteins having mannose -mannose linkages in the form of a-l $\rightarrow$3 or a-l $\rightarrow$6 mannose-mannose linkages.

[00186] The binding agent can be an agent that binds one or more lectins. Lectin capture can be applied to the isolation of the biomarker cathepsin D since it is a glycosylated protein capable of binding the lectins Galanthus nivalis agglutinin (GNA) and concanavalin A (ConA).

[00187] Methods and devices for using lectins to capture vesicles are described in International Patent Applications PCT/US20 10/05 8461, entitled "METHODS AND SYSTEMS FOR ISOLATING, STORING, AND ANALYZING VESICLES" and filed November 30, 2010; PCT/US2009/066626, entitled "AFFINITY CAPTURE OF CIRCULATING BIOMARKERS" and filed December 3, 2009; PCT/US20 10/03 7467, entitled "METHODS AND MATERIALS FOR ISOLATING EXOSOMES" and filed June 4, 2010; and PCT/US2007/006101, entitled "EXTRACORPOREAL REMOVAL OF MICROVESICULAR PARTICLES" and filed March 9, 2007, each of which applications is incorporated by reference herein in its entirety.

[00188] The binding agent can be an antibody. For example, a vesicle may be isolated using one or more antibodies specific for one or more antigens present on the vesicle. For example, a vesicle can have CD63 on its

PREOMICS EXHIBIT 1002
Page 976 of 1861

surface, and an antibody, or capture antibody, for CD63 can be used to isolate the vesicle. Alternatively, a vesicle derived from a tumor cell can express EpCam, the vesicle can be isolated using an antibody for EpCam and CD63. Other antibodies for isolating vesicles can include an antibody, or capture antibody, to CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, or 5T4. Other antibodies for isolating vesicles can include an antibody, or capture antibody, to DR3, STEAP, epha2, TMEM21 1, MFG-E8, Tissue Factor (TF), unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, or TETS.

[00189] In some embodiments, the capture agent is an antibody to CD9, CD63, CD81, PSMA, PCSA, B7H3, EpCam, PSCA, ICAM, STEAP, or EGFR. The capture agent can also be used to identify a biomarker of a vesicle. For example, a capture agent such as an antibody to CD9 would identify CD9 as a biomarker of the vesicle. In some embodiments, a plurality of capture agents can be used, such as in multiplex analysis. The plurality of captures agents can comprise binding agents to one or more of: CD9, CD63, CD81, PSMA, PCSA, B7H3, EpCam, PSCA, ICAM, STEAP, and EGFR. In some embodiments, the plurality of capture agents comprise binding agents to CD9, CD63, CD81, PSMA, PCSA, B7H3, MFG-E8, and/or EpCam. In yet other embodiments, the plurality of capture agents comprises binding agents to CD9, CD63, CD81, PSMA, PCSA, B7H3, EpCam, PSCA, ICAM, STEAP, and/or EGFR. The plurality of capture agents comprises binding agents to TMEM21 1, MFG-E8, Tissue Factor (TF), and/or CD24.

[00190] The antibodies referenced herein can be immunoglobulin molecules or immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen and synthetic antibodies. The immunoglobulin molecules can be of any class (e.g., IgG, IgE, IgM, IgD or IgA) or subclass of immunoglobulin molecule. Antibodies include, but are not limited to, polyclonal, monoclonal, bispecific, synthetic, humanized and chimeric antibodies, single chain antibodies, Fab fragments and F(ab')2 fragments, Fv or Fv' portions, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, or epitope-binding fragments of any of the above. An antibody, or generally any molecule, "binds specifically" to an antigen (or other molecule) if the antibody binds preferentially to the antigen, and, e.g., has less than about 30%, 20%, 10%, 5% or 1% cross-reactivity with another molecule.

[00191] The binding agent can also be a polypeptide or peptide. Polypeptide is used in its broadest sense and may include a sequence of subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. The polypeptides may be naturally occurring, processed forms of naturally occurring polypeptides (such as by enzymatic digestion), chemically synthesized or recombinantly expressed. The polypeptides for use in the methods of the present invention may be chemically synthesized using standard techniques. The polypeptides may comprise D-amino acids (which are resistant to L- amino acid-specific proteases), a combination of D- and L-amino acids, β amino acids, or various other designer or non-naturally occurring amino acids (e.g., β-methyl amino acids, Ca- methyl amino acids, and Na-methyl amino acids, etc.) to convey special properties. Synthetic amino acids may include ornithine for lysine, and norleucine for leucine or isoleucine. In addition, the polypeptides can have peptidomimetic bonds, such as ester bonds, to prepare polypeptides with novel properties. For example, a polypeptide may be generated that incorporates a reduced peptide bond, i.e., R_1-CH_2-NH-R_2, where R_1 and R_2 are amino acid residues or sequences. A reduced peptide bond may be introduced as a dipeptide subunit. Such a polypeptide would be resistant to protease activity, and would possess an extended half- live in vivo. Polypeptides can also include peptoids (N-substituted glycines), in which the side chains are appended to nitrogen atoms along the molecule's backbone, rather than to the a-

PREOMICS EXHIBIT 1002
Page 977 of 1861

carbons, as in amino acids. Polypeptides and peptides are intended to be used interchangeably throughout this application, i.e. where the term peptide is used, it may also include polypeptides and where the term polypeptides is used, it may also include peptides. The term "protein" is also intended to be used interchangeably throughout this application with the terms "polypeptides" and "peptides" unless otherwise specified.

[00192] A vesicle may be isolated, captured or detected using a binding agent. The binding agent can be an agent that binds a vesicle "housekeeping protein," or general vesicle biomarker. The biomarker can be CD63, CD9, CD81, CD82, CD37, CD53, Rab-5b, Annexin V or MFG-E8. Tetraspanins, a family of membrane proteins with four transmembrane domains, can be used as general vesicle markers. The tetraspanins include CD151, CD53, CD37, CD82, CD81, CD9 and CD63. There have been over 30 tetraspanins identified in mammals, including the TSPAN1 (TSP-1), TSPAN2 (TSP-2), TSPAN3 (TSP-3), TSPAN4 (TSP-4, NAG-2), TSPAN5 (TSP-5), TSPAN6 (TSP-6), TSPAN7 (CD231, TALLA-1, A15), TSPAN8 (CO-029), TSPAN9 (NET-5), TSPAN10 (Oculospanin), TSPAN11 (CD151-like), TSPAN12 (NET-2), TSPAN13 (NET-6), TSPAN14, TSPAN15 (NET-7), TSPAN16 (TM4-B), TSPAN17, TSPAN18, TSPAN19, TSPAN20 (UPlb, UPK1B), TSPAN21 (UPla, UPK1A), TSPAN22 (RDS, PRPH2), TSPAN23 (ROM1), TSPAN24 (CD151), TSPAN25 (CD53), TSPAN26 (CD37), TSPAN27 (CD82), TSPAN28 (CD81), TSPAN29 (CD9), TSPAN30 (CD63), TSPAN31 (SAS), TSPAN32 (TSSC6), TSPAN33, and TSPAN34. Other commonly observed vesicle markers include those listed in **Table 3.** Any of these proteins can be used as vesicle markers. Furthermore, any of the markers disclosed herein or in **Table 3** can be selected in identifying a candidate biosignature for a disease or condition, where the one or more selected biomarkers have a direct or indirect role or function in mechanisms involved in the disease or condition.

Table 3: Proteins Observed in Vesicles from Multiple Cell Types

Class	Protein
Antigen Presentation	MHC class I, MHC class II, Integrins, Alpha 4 beta 1, Alpha M beta 2, Beta 2
Immunoglobulin family	ICAM1/CD54, P-selection
Cell-surface peptidases	Dipeptidylpeptidase IV/CD26, Aminopeptidase n/CD13
Tetraspanins	CD151, CD53, CD37, CD82, CD81, CD9 and CD63
Heat-shock proteins	Hsp70, Hsp84/90
Cytoskeletal proteins	Actin, Actin-binding proteins, Tubulin
Membrane transport and fusion	Annexin I, Annexin II, Annexin IV, Annexin V, Annexin VI, RAB7/RAP1B/RADGDI
Signal transduction	Gi2alpha/14-3-3, CBL/LCK
Abundant membrane proteins	CD63, GAPDH, CD9, CD81, ANXA2, ENO1, SDCBP, MSN, MFGE8, EZR, GK, ANXA1, LAMP2, DPP4, TSG101, HSPA1A, GDI2, CLTC, LAMP1, Cd86, ANPEP, TFRC, SLC3A2, RDX, RAP1B, RAB5C, RAB5B, MYH9, ICAM1, FN1, RAB11B, PIGR, LGALS3, ITGB1, EHD1, CLIC1, ATP1A1, ARF1, RAP1A, P4HB, MUC1, KRT10, HLA-A, FLOT1, CD59, C1orf58, BASP1, TACSTD1, STOM
Other Transmembrane Proteins	Cadherins: CDH1, CDH2, CDH12, CDH3, Deomoglein, DSG1, DSG2, DSG3, DSG4, Desmocollin, DSC1, DSC2, DSC3, Protocadherins, PCDH1, PCDH10, PCDH11x, PCDH11y, PCDH12, FAT, FAT2, FAT4, PCDH15, PCDH17, PCDH18, PCDH19; PCDH20; PCDH7, PCDH8, PCDH9, PCDHA1, PCDHA10, PCDHA11, PCDHA12, PCDHA13, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8, PCDHA9, PCDHAC1, PCDHAC2, PCDHB1, PCDHB10, PCDHB11, PCDHB12, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB17, PCDHB18, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHB7, PCDHB8, PCDHB9, PCDHGA1, PCDHGA10, PCDHGA11, PCDHGA12, PCDHGA2; PCDHGA3, PCDHGA4, PCDHGA5, PCDHGA6, PCDHGA7, PCDHGA8, PCDHGA9,

PREOMICS EXHIBIT 1002
Page 978 of 1861

	PCDHGB1, PCDHGB2, PCDHGB3, PCDHGB4, PCDHGB5, PCDHGB6, PCDHGB7, PCDHGC3, PCDHGC4, PCDHGC5, CDH9 (cadherin 9, type 2 (Tl-cadherin)), CDH10 (cadherin 10, type 2 (T2-cadherin)), CDH5 (VE-cadherin (vascular endothelial)), CDH6 (K-cadherin (kidney)), CDH7 (cadherin 7, type 2), CDH8 (cadherin 8, type 2), CDH1 1 (OB-cadherin (osteoblast)), CDH13 (T-cadherin - H-cadherin (heart)), CDH15 (M-cadherin (myotubule)), CDH16 (KSP-cadherin), CDH17 (LI cadherin (liver-intestine)), CDH18 (cadherin 18, type 2), CDH19 (cadherin 19, type 2), CDH20 (cadherin 20, type 2), CDH23 (cadherin 23, (neurosensory epithelium)), CDH10, CDH1 1, CDH13, CDH15, CDH16, CDH17, CDH1 8, CDH19, CDH20, CDH22, CDH23, CDH24, CDH26, CDH28, CDH4, CDH5, CDH6, CDH7, CDH8, CDH9, CELSR1, CELSR2, CELSR3, CLSTN1, CLSTN2, CLSTN3, DCHS1, DCHS2, LOC3891 18, PCLKC, RESDA1, RET

[00193] The binding agent can also be an agent that binds to a vesicle derived from a specific cell type, such as a tumor cell (e.g. binding agent for Tissue factor, EpCam, B7H3, RAGE or CD24) or a specific cell-of-origin. The binding agent used to isolate or detect a vesicle can be a binding agent for an antigen selected from **Fig. 1** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein. The binding agent for a vesicle can also be selected from those listed in **Fig. 2** of International Patent Application Serial No. PCT/US201 1/03 1479. The binding agent can be for an antigen such as a tetraspanin, MFG-E8, Annexin V, 5T4, B7H3, caveolin, CD63, CD9, E-Cadherin, Tissue factor, MFG-E8, TMEM21 1, CD24, PSCA, PCSA, PSMA, Rab-5B, STEAP, TNFR1, CD81, EpCam, CD59, CD81, ICAM, EGFR, or CD66. A binding agent for a platelet can be a glycoprotein such as GpIa-IIa, GpIIb-IIIa, GpIIIb, Gplb, or GpIX. A binding agent can be for an antigen comprisine one or more of CD9, Erb2, Erb4, CD81, Erb3, MUC16, CD63, DLL4, HLA-Drpe, B7H3, IFNAR, 5T4, PCSA, MICB, PSMA, MFG-E8, Mucl, PSA, Muc2, Unc93a, VEGFR2, EpCAM, VEGF A, TMPRSS2, RAGE, PSCA, CD40, Mucl 7, IL-17-RA, and CD80. For example, the binding agent can be one or more of CD9, CD63, CD81, B7H3, PCSA, MFG-E8, MUC2, EpCam, RAGE and Mucl7. One or more binding agents, such as one or more binding agents for two or more of the antigens, can be used for isolating or detecting a vesicle. The binding agent used can be selected based on the desire of isolating or detecting a vesicle derived from a particular cell type or cell-of-origin specific vesicle.

[00194] A binding agent can also be linked directly or indirectly to a solid surface or substrate. A solid surface or substrate can be any physically separable solid to which a binding agent can be directly or indirectly attached including, but not limited to, surfaces provided by microarrays and wells, particles such as beads, columns, optical fibers, wipes, glass and modified or functionalized glass, quartz, mica, diazotized membranes (paper or nylon), polyformaldehyde, cellulose, cellulose acetate, paper, ceramics, metals, metalloids, semiconductive materials, quantum dots, coated beads or particles, other chromatographic materials, magnetic particles; plastics (including acrylics, polystyrene, copolymers of styrene or other materials, polypropylene, polyethylene, polybutylene, polyurethanes, polytetrafluoroethylene (PTFE, Teflon®), etc.), polysaccharides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses, plastics, ceramics, conducting polymers (including polymers such as polypyrole and polyindole); micro or nanostructured surfaces such as nucleic acid tiling arrays, nanotube, nanowire, or nanoparticulate decorated surfaces; or porous surfaces or gels such as methacrylates, acrylamides, sugar polymers, cellulose, silicates, or other fibrous or stranded polymers. In addition, as is known the art, the substrate may be coated using passive or chemically-derivatized coatings with any number of materials,

PREOMICS EXHIBIT 1002
Page 979 of 1861

including polymers, such as dextrans, acrylamides, gelatins or agarose. Such coatings can facilitate the use of the array with a biological sample.

[00195] For example, an antibody used to isolate a vesicle can be bound to a solid substrate such as a well, such as commercially available plates (e.g. from Nunc, Milan Italy). Each well can be coated with the antibody. In some embodiments, the antibody used to isolate a vesicle is bound to a solid substrate such as an array. The array can have a predetermined spatial arrangement of molecule interactions, binding islands, biomolecules, zones, domains or spatial arrangements of binding islands or binding agents deposited within discrete boundaries. Further, the term array may be used herein to refer to multiple arrays arranged on a surface, such as would be the case where a surface bore multiple copies of an array. Such surfaces bearing multiple arrays may also be referred to as multiple arrays or repeating arrays.

[00196] Arrays typically contain addressable moieties that can detect the presense of an entity, e.g., a vesicle in the sample via a binding event. An array may be referred to as a microarray. Arrays or microarrays include without limitation DNA microarrays, such as cDNA microarrays, oligonucleotide microarrays and SNP microarrays, microRNA arrays, protein microarrays, antibody microarrays, tissue microarrays, cellular microarrays (also called transfection microarrays), chemical compound microarrays, and carbohydrate arrays (glycoarrays). DNA arrays typically comprise addressable nucleotide sequences that can bind to sequences present in a sample. MicroRNA arrays, e.g., the MMChips array from the University of Louisville or commercial systems from Agilent, can be used to detect microRNAs. Protein microarrays can be used to identify protein-protein interactions, including without limitation identifying substrates of protein kinases, transcription factor protein-activation, or to identify the targets of biologically active small molecules. Protein arrays may comprise an array of different protein molecules, commonly antibodies, or nucleotide sequences that bind to proteins of interest. In a non-limiting example, a protein array can be used to detect vesicles having certain proteins on their surface. Antibody arrays comprise antibodies spotted onto the protein chip that are used as capture molecules to detect proteins or other biological materials from a sample, e.g., from cell or tissue lysate solutions. For example, antibody arrays can be used to detect vesicle-associated biomarkers from bodily fluids, e.g., serum or urine. Tissue microarrays comprise separate tissue cores assembled in array fashion to allow multiplex histological analysis. Cellular microarrays, also called transfection microarrays, comprise various capture agents, such as antibodies, proteins, or lipids, which can interact with cells to facilitate their capture on addressable locations. Cellular arrays can also be used to capture vesicles due to the similarity between a vesicle and cellular membrane. Chemical compound microarrays comprise arrays of chemical compounds and can be used to detect protein or other biological materials that bind the compounds. Carbohydrate arrays (glycoarrays) comprise arrays of carbohydrates and can detect, e.g., protein that bind sugar moieties. One of skill will appreciate that similar technologies or improvements can be used according to the methods of the invention.

[00197] A binding agent can also be bound to particles such as beads or microspheres. For example, an antibody specific for a component of a vesicle can be bound to a particle, and the antibody-bound particle is used to isolate a vesicle from a biological sample. In some embodiments, the microspheres may be magnetic or fluorescently labeled. In addition, a binding agent for isolating vesicles can be a solid substrate itself. For example, latex beads, such as aldehyde/sulfate beads (Interfacial Dynamics, Portland, OR) can be used.

PREOMICS EXHIBIT 1002
Page 980 of 1861

[00198] A binding agent bound to a magnetic bead can also be used to isolate a vesicle. For example, a biological sample such as serum from a patient can be collected for colon cancer screening. The sample can be incubated with anti-CCSA-3 (Colon Cancer-Specific Antigen) coupled to magnetic microbeads. A low-density microcolumn can be placed in the magnetic field of a MACS Separator and the column is then washed with a buffer solution such as Tris-buffered saline. The magnetic immune complexes can then be applied to the column and unbound, non-specific material can be discarded. The CCSA-3 selected vesicle can be recovered by removing the column from the separator and placing it on a collection tube. A buffer can be added to the column and the magnetically labeled vesicle can be released by applying the plunger supplied with the column. The isolated vesicle can be diluted in IgG elution buffer and the complex can then be centrifuged to separate the microbeads from the vesicle. The pelleted isolated cell-of-origin specific vesicle can be resuspended in buffer such as phosphate -buffered saline and quantitated. Alternatively, due to the strong adhesion force between the antibody captured cell-of-origin specific vesicle and the magnetic microbeads, a proteolytic enzyme such as trypsin can be used for the release of captured vesicles without the need for centrifugation. The proteolytic enzyme can be incubated with the antibody captured cell-of-origin specific vesicles for at least a time sufficient to release the vesicles.

[00199] A binding agent, such as an antibody, for isolating vesicles is preferably contacted with the biological sample comprising the vesicles of interest for at least a time sufficient for the binding agent to bind to a component of the vesicle. For example, an antibody may be contacted with a biological sample for various intervals ranging from seconds days, including but not limited to, about 10 minutes, 30 minutes, 1 hour, 3 hours, 5 hours, 7 hours, 10 hours, 15 hours, 1 day, 3 days, 7 days or 10 days.

[00200] A binding agent, such as an antibody specific to an antigen listed in **Fig. 1** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein, or a binding agent listed in **Fig. 2** of International Patent Application Serial No. PCT/US201 1/03 1479, can be labeled to facilitate detection. Appropriate labels include without limitation a magnetic label, a fluorescent moiety, an enzyme, a chemiluminescent probe, a metal particle, a non-metal colloidal particle, a polymeric dye particle, a pigment molecule, a pigment particle, an electrochemically active species, semiconductor nanocrystal or other nanoparticles including quantum dots or gold particles, fluorophores, quantum dots, or radioactive labels. Protein labels include green fluorescent protein (GFP) and variants thereof (e.g., cyan fluorescent protein and yellow fluorescent protein); and luminescent proteins such as luciferase, as described below. Radioactive labels include without limitation radioisotopes (radionuclides), such as ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, or ^{213}Bi. Fluorescent labels include without limitation a rare earth chelate (e.g., europium chelate), rhodamine; fluorescein types including without limitation FITC, 5-carboxyfluorescein, 6-carboxy fluorescein; a rhodamine type including without limitation TAMRA; dansyl; Lissamine; cyanines; phycoerythrins; Texas Red; Cy3, Cy5, dapoxyl, NBD, Cascade Yellow, dansyl, PyMPO, pyrene, 7-diethylaminocoumarin-3-carboxylic acid and other coumarin derivatives, Marina Blue™, Pacific Blue™, Cascade Blue™, 2-anthracenesulfonyl, PyMPO, 3,4,9, 10-perylene-tetracarboxylic acid, 2,7-difluorofluorescein (Oregon Green™ 488-X), 5-carboxyfluorescein, Texas Red™-X, Alexa Fluor 430, 5-carboxytetramethybhodamine (5-TAMRA), 6-carboxytetramethylrhodamine (6-TAMRA), BODIPY FL, bimane, and Alexa Fluor 350, 405, 488, 500, 5 14, 532, 546, 555, 568, 594, 610, 633, 647, 660, 680, 700, and

PREOMICS EXHIBIT 1002
Page 981 of 1861

750, and derivatives thereof, among many others. See, e.g., "The Handbook—A Guide to Fluorescent Probes and Labeling Technologies," Tenth Edition, available on the internet at probes (dot) invitrogen (dot) com/handbook. The fluorescent label can be one or more of FAM, dRHO, 5-FAM, 6FAM, dR6G, JOE, HEX, VIC, TET, dTAMRA, TAMRA, NED, dROX, PET, BHQ, Gold540 and LIZ.

[00201] A binding agent can be directly or indirectly labeled, e.g., the label is attached to the antibody through biotin-streptavidin. Alternatively, an antibody is not labeled, but is later contacted with a second antibody that is labeled after the first antibody is bound to an antigen of interest.

[00202] For example, various enzyme-substrate labels are available or disclosed (see for example, U.S. Pat. No. 4,275,149). The enzyme generally catalyzes a chemical alteration of a chromogenic substrate that can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Examples of enzyme-substrate combinations include, but are not limited to, horseradish peroxidase (HRP) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethylbenzidine hydrochloride (TMB)); alkaline phosphatase (AP) with para-nitrophenyl phosphate as chromogenic substrate; and β-D-galactosidase (β-D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl- β-D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl^-D-galactosidase.

[00203] Depending on the method of isolation or detection used, the binding agent may be linked to a solid surface or substrate, such as arrays, particles, wells and other substrates described above. Methods for direct chemical coupling of antibodies, to the cell surface are known in the art, and may include, for example, coupling using glutaraldehyde or maleimide activated antibodies. Methods for chemical coupling using multiple step procedures include biotinylation, coupling of trinitrophenol (TNP) or digoxigenin using for example succinimide esters of these compounds. Biotinylation can be accomplished by, for example, the use of D-biotinyl-N-hydroxysuccinimide. Succinimide groups react effectively with amino groups at pH values above 7, and preferentially between about pH 8.0 and about pH 8.5. Biotinylation can be accomplished by, for example, treating the cells with dithiothreitol followed by the addition of biotin maleimide.

Particle-based Assays

[00204] As an alternative to planar arrays, assays using particles, such as bead based assays, are capable of use with a binding agent. For example, antibodies or aptamers are easily conjugated with commercially available beads. *See, e.g.,* Fan *et al* , Illumina universal bead arrays. Methods Enzymol. 2006 410:57-73; Srinivas *et al.* Anal. Chem. 201 1 Oct. 2 1, *Aptamerfunctionalized Microgel Particlesfor Protein Detection;* See also, review article on aptamers as therapeutic and diagnostic agents, Brody and Gold, Rev. Mol. Biotech. 2000, 74:5-13.

[00205] Multiparametric assays or other high throughput detection assays using bead coatings with cognate ligands and reporter molecules with specific activities consistent with high sensitivity automation can be used. In a bead based assay system, a binding agent for a biomarker or vesicle, such as a capture agent (e.g. capture

PREOMICS EXHIBIT 1002
Page 982 of 1861

antibody), can be immobilized on an addressable microsphere. Each binding agent for each individual binding assay can be coupled to a distinct type of microsphere (i.e., microbead) and the assay reaction takes place on the surface of the microsphere, such as depicted in **FIG. 2B.** A binding agent for a vesicle can be a capture antibody or aptamer coupled to a bead. Dyed microspheres with discrete fluorescence intensities are loaded separately with their appropriate binding agent or capture probes. The different bead sets carrying different binding agents can be pooled as necessary to generate custom bead arrays. Bead arrays are then incubated with the sample in a single reaction vessel to perform the assay. *See* **FIGs. 8C-D** for illustrative methods of detecting microvesicles using microbeads with antibody binding agents.

[00206] A bead substrate can provide a platform for attaching one or more binding agents, including aptamer(s) or antibodies. One of skill will appreciate that the illustrative schemes shown in **FIGs. 8C-D** can employ aptamers along with or instead of antibodies. For multiplexing, multiple different bead sets (e.g., those commercially available from Illumina, Inc., San Diego, CA, USA, or Luminex Corporation, Austin, TX, USA) can have different binding agents which are specific to different target molecules. Beads can also be used for different purposes, e.g., detection and/or isolation. For example, a bead can be conjugated to an aptamer used to detect the presence (quantitatively or qualitatively) of a given biomarker, or it can also be used to isolate a component present in a selected biological sample (e.g., cell, cell-fragment or vesicle comprising the target molecule to which the binding agent is configured to bind or associate). Various molecules of organic origin can be conjugated to a microbeads, e.g., polysterene beads, through use of commercially available kits. One of skill will appreciate that an assay can use multiple types of binding agents. For example, a bead may be conjugated to an aptamer which serves to bind and capture a biomarker, and a labeled antibody can be used to further detect the captured biomarker. Similarly, a bead may be conjugated to an antibody which serves to bind and capture a biomarker, and a labeled aptamer can be used to further detect the captured biomarker. Any such useful combination of binding agents are contemplated by the invention.

[00207] One or more binding agent can be used with any bead based substrate, including but not limited to magnetic capture method, fluorescence activated cell sorting (FACS) or laser cytometry. Magnetic capture methods can include, but are not limited to, the use of magnetically activated cell sorter (MACS) microbeads or magnetic columns. Examples of bead or particle based methods that can be used in the methods of the invention include the bead systems described in any of U.S. Patent Nos. 4,55 1,435, 4,795,698, 4,925,788, 5,108,933, 5,1 86,827, 5,200,084 or 5,158,871 ; 7,399,632; 8,124,015; 8,008,019; 7,955,802; 7,445,844; 7,274,3 16; 6,773,812; 6,623,526; 6,599,33 1; 6,057,107; 5,736,330; or International Patent Application Nos. PCT/US20 12/425 19; PCT/US 1993/04 145.

Flow Cytometry

[00208] Isolation or detection of a vesicle using a particle such as a bead or microsphere can also be performed using flow cytometry. Flow cytometry can be used for sorting microscopic particles suspended in a stream of fluid. As particles pass through they can be selectively charged and on their exit can be deflected into separate paths of flow. It is therefore possible to separate populations from an original mix, such as a biological sample, with a high degree of accuracy and speed. Flow cytometry allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of single cells flowing through an optical/electronic detection apparatus. A beam of light, usually laser light, of a single frequency (color) is directed onto a hydrodynamically focused stream of fluid. A number of detectors are aimed at the point where the stream passes through the light beam;

PREOMICS EXHIBIT 1002
Page 983 of 1861

one in line with the light beam (Forward Scatter or FSC) and several perpendicular to it (Side Scatter or SSC) and one or more fluorescent detectors.

[00209] Each suspended particle passing through the beam scatters the light in some way, and fluorescent chemicals in the particle may be excited into emitting light at a lower frequency than the light source. This combination of scattered and fluorescent light is picked up by the detectors, and by analyzing fluctuations in brightness at each detector (one for each fluorescent emission peak), it is possible to deduce various facts about the physical and chemical structure of each individual particle. FSC correlates with the cell size and SSC depends on the inner complexity of the particle, such as shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness. Some flow cytometers have eliminated the need for fluorescence and use only light scatter for measurement.

[00210] Flow cytometers can analyze several thousand particles every second in "real time" and can actively separate out and isolate particles having specified properties. They offer high-throughput automated quantification, and separation, of the set parameters for a high number of single cells during each analysis session. Flow cytomers can have multiple lasers and fluorescence detectors, allowing multiple labels to be used to more precisely specify a target population by their phenotype. Thus, a flow cytometer, such as a multicolor flow cytometer, can be used to detect one or more vesicles with multiple fluorescent labels or colors. In some embodiments, the flow cytometer can also sort or isolate different vesicle populations, such as by size or by different markers.

[00211] The flow cytometer may have one or more lasers, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more lasers. In some embodiments, the flow cytometer can detect more than one color or fluorescent label, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 different colors or fluorescent labels. For example, the flow cytometer can have at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 fluorescence detectors.

[00212] Examples of commercially available flow cytometers that can be used to detect or analyze one or more vesicles, to sort or separate different populations of vesicles, include, but are not limited to the MoFlo™ XDP Cell Sorter (Beckman Coulter, Brea, CA), MoFlo™ Legacy Cell Sorter (Beckman Coulter, Brea, CA), BD FACSAria™ Cell Sorter (BD Biosciences, San Jose, CA), BD™ LSRII (BD Biosciences, San Jose, CA), and BD FACSCalibur™ (BD Biosciences, San Jose, CA). Use of multicolor or multi-fluor cytometers can be used in multiplex analysis of vesicles, as further described below. In some embodiments, the flow cytometer can sort, and thereby collect or sort more than one population of vesicles based one or more characteristics. For example, two populations of vesicles differ in size, such that the vesicles within each population have a similar size range and can be differentially detected or sorted. In another embodiment, two different populations of vesicles are differentially labeled.

[00213] The data resulting from flow-cytometers can be plotted in 1 dimension to produce histograms or seen in 2 dimensions as dot plots or in 3 dimensions with newer software. The regions on these plots can be sequentially separated by a series of subset extractions which are termed gates. Specific gating protocols exist for diagnostic and clinical purposes especially in relation to hematology. The plots are often made on logarithmic scales. Because different fluorescent dye's emission spectra overlap, signals at the detectors have to be compensated electronically as well as computationally. Fluorophores for labeling biomarkers may include

PREOMICS EXHIBIT 1002
Page 984 of 1861

those described in *Ormerod, Flow Cytometry 2nd ed., Springer-Verlag, New York (1999),* and in *Nida et ah, Gynecologic Oncology 2005;4 889-894* which is incorporated herein by reference.

[00214] In various embodiments of the invention, flow cytometry is used to assess a microvesicle population in a biological sample. If desired, the microvesicle population can be sorted from other particles (e.g., cell debris, protein aggregates, etc) in a sample by labeling the vesicles using one or more general vesicle marker. The general vesicle marker can be a marker in **Table 3.** Commonly used vesicle markers include tetraspanins such as CD9, CD63 and/or CD81 . Vesicles comprising one or more tetraspanin are sometimes refered to as "Tet+" herein to indicate that the vesicles are tetraspanin-positive. The sorted microvesicles can be further assessed using methodology described herein. E.g., surface antigens on the sorted microvesicles can be detected using flow or other methods. In some embodiments, payload within the sorted microvesicles is assessed. As an illustrative example, a population of microvesicles is contacted with a labeled binding agent to a surface antigen of interest, the contacted microvesicles are sorted using flow cytometry, and payload with the microvesicles is assessed. The payload may be polypeptides, nucleic acids (e.g., mRNA or microRNA) or other biological entities as desired. Such assessment is used to characterize a phenotype as described herein, e.g., to diagnose, prognose or theranose a cancer.

[00215] In an embodiment, flow sorting is used to distinguish microvesicle populations from other biological complexes. In a non-limiting example, Ago2+/Tet+ and Ago2+/Tet- particles are detected using flow methodology to separate Ago2+ vesicles from vesicle-free Ago2+ complexes, respectively.

Multiplexing

[00216] Multiplex experiments comprise experiments that can simultaneously measure multiple analytes in a single assay. Vesicles and associated biomarkers can be assessed in a multiplex fashion. Different binding agents can be used for multiplexing different circulating biomarkers, e.g., microRNA, protein, or vesicle populations. Different biomarkers, e.g., different vesicle populations, can be isolated or detected using different binding agents. Each population in a biological sample can be labeled with a different signaling label, such as a fluorophore, quantum dot, or radioactive label, such as described above. The label can be directly conjugated to a binding agent or indirectly used to detect a binding agent that binds a vesicle. The number of populations detected in a multiplexing assay is dependent on the resolution capability of the labels and the summation of signals, as more than two differentially labeled vesicle populations that bind two or more affinity elements can produce summed signals.

[00217] Multiplexing of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 different circulating biomarkers may be performed. For example, one population of vesicles specific to a cell-of-origin can be assayed along with a second population of vesicles specific to a different cell-of-origin, where each population is labeled with a different label. Alternatively, a population of vesicles with a particular biomarker or biosignature can be assayed along with a second population of vesicles with a different biomarker or biosignature. In some cases, hundreds or thousands of vesicles are assessed in a single assay.

[00218] In one embodiment, multiplex analysis is performed by applying a plurality of vesicles comprising more than one population of vesicles to a plurality of substrates, such as beads. Each bead is coupled to one or more capture agents. The plurality of beads is divided into subsets, where beads with the same capture agent or combination of capture agents form a subset of beads, such that each subset of beads has a different capture agent or combination of capture agents than another subset of beads. The beads can then be used to capture

PREOMICS EXHIBIT 1002
Page 985 of 1861

vesicles that comprise a component that binds to the capture agent. The different subsets can be used to capture different populations of vesicles. The captured vesicles can then be analyzed by detecting one or more biomarkers.

[00219] Flow cytometry can be used in combination with a particle-based or bead based assay. Multiparametric immunoassays or other high throughput detection assays using bead coatings with cognate ligands and reporter molecules with specific activities consistent with high sensitivity automation can be used. For example, beads in each subset can be differentially labeled from another subset. In a particle based assay system, a binding agent or capture agent for a vesicle, such as a capture antibody, can be immobilized on addressable beads or microspheres. Each binding agent for each individual binding assay (such as an immunoassay when the binding agent is an antibody) can be coupled to a distinct type of microsphere (i.e., microbead) and the binding assay reaction takes place on the surface of the microspheres. Microspheres can be distinguished by different labels, for example, a microsphere with a specific capture agent would have a different signaling label as compared to another microsphere with a different capture agent. For example, microspheres can be dyed with discrete fluorescence intensities such that the fluorescence intensity of a microsphere with a specific binding agent is different than that of another microsphere with a different binding agent. Biomarkers bound by different capture agents can be differentially detected using different labels.

[00220] A microsphere can be labeled or dyed with at least 2 different labels or dyes. In some embodiments, the microsphere is labeled with at least 3, 4, 5, 6, 7, 8, 9, or 10 different labels. Different microspheres in a plurality of microspheres can have more than one label or dye, wherein various subsets of the microspheres have various ratios and combinations of the labels or dyes permitting detection of different microspheres with different binding agents. For example, the various ratios and combinations of labels and dyes can permit different fluorescent intensities. Alternatively, the various ratios and combinations maybe used to generate different detection patters to identify the binding agent. The microspheres can be labeled or dyed externally or may have intrinsic fluorescence or signaling labels. Beads can be loaded separately with their appropriate binding agents and thus, different vesicle populations can be isolated based on the different binding agents on the differentially labeled microspheres to which the different binding agents are coupled.

[00221] In another embodiment, multiplex analysis can be performed using a planar substrate, wherein the substrate comprises a plurality of capture agents. The plurality of capture agents can capture one or more populations of vesicles, and one or more biomarkers of the captured vesicles detected. The planar substrate can be a microarray or other substrate as further described herein.

Binding Agents

[00222] A vesicle may be isolated or detected using a binding agent for a novel component of a vesicle, such as an antibody for a novel antigen specific to a vesicle of interest. Novel antigens that are specific to a vesicle of interest may be isolated or identified using different test compounds of known composition bound to a substrate, such as an array or a plurality of particles, which can allow a large amount of chemical/structural space to be adequately sampled using only a small fraction of the space. The novel antigen identified can also serve as a biomarker for the vesicle. For example, a novel antigen identified for a cell-of-origin specific vesicle can be a useful biomarker.

[00223] The term "agent" or "reagent" as used in respect to contacting a sample can mean any entity designed to bind, hybridize, associate with or otherwise detect or facilitate detection of a target molecule, including target

PREOMICS EXHIBIT 1002
Page 986 of 1861

polypeptides, peptides, nucleic acid molecules, leptins, lipids, or any other biological entity that can be detected as described herein or as known in the art. Examples of such agents/reagents are well known in the art, and include but are not limited to universal or specific nucleic acid primers, nucleic acid probes, antibodies, aptamers, peptoid, peptide nucleic acid, locked nucleic acid, lectin, dendrimer, chemical compound, or other entities described herein or known in the art.

[00224] A binding agent can be identified by screening either a homogeneous or heterogeneous vesicle population against test compounds. Since the composition of each test compound on the substrate surface is known, this constitutes a screen for affinity elements. For example, a test compound array comprises test compounds at specific locations on the substrate addressable locations, and can be used to identify one or more binding agents for a vesicle. The test compounds can all be unrelated or related based on minor variations of a core sequence or structure. The different test compounds may include variants of a given test compound (such as polypeptide isoforms), test compounds that are structurally or compositionally unrelated, or a combination thereof.

[00225] A test compound can be a peptoid, polysaccharide, organic compound, inorganic compound, polymer, lipids, nucleic acid, polypeptide, antibody, protein, polysaccharide, or other compound. The test compound can be natural or synthetic. The test compound can comprise or consist of linear or branched heteropolymeric compounds based on any of a number of linkages or combinations of linkages (e.g., amide, ester, ether, thiol, radical additions, metal coordination, etc.), dendritic structures, circular structures, cavity structures or other structures with multiple nearby sites of attachment that serve as scaffolds upon which specific additions are made. Thes test compound can be spotted on a substrate or synthesized in situ, using standard methods in the art. In addition, the test compound can be spotted or synthesized in situ in combinations in order to detect useful interactions, such as cooperative binding.

[00226] The test compound can be a polypeptide with known amino acid sequence, thus, detection of a test compound binding with a vesicle can lead to identification of a polypeptide of known amino sequence that can be used as a binding agent. For example, a homogenous population of vesicles can be applied to a spotted array on a slide containing between a few and 1,000,000 test polypeptides having a length of variable amino acids. The polypeptides can be attached to the surface through the C-terminus. The sequence of the polypeptides can be generated randomly from 19 amino acids, excluding cysteine. The binding reaction can include a non-specific competitor, such as excess bacterial proteins labeled with another dye such that the specificity ratio for each polypeptide binding target can be determined. The polypeptides with the highest specificity and binding can be selected. The identity of the polypeptide on each spot is known, and thus can be readily identified. Once the novel antigens specific to the homogeneous vesicle population, such as a cell-of-origin specific vesicle is identified, such cell-of-origin specific vesicles may subsequently be isolated using such antigens in methods described hereafter.

[00227] An array can also be used for identifying an antibody as a binding agent for a vesicle. Test antibodies can be attached to an array and screened against a heterogeneous population of vesicles to identify antibodies that can be used to isolate or identify a vesicle. A homogeneous population of vesicles such as cell-of-origin specific vesicles can also be screened with an antibody array. Other than identifying antibodies to isolate or detect a homogeneous population of vesicles, one or more protein biomarkers specific to the homogenous population can be identified. Commercially available platforms with test antibodies pre-selected or custom

PREOMICS EXHIBIT 1002
Page 987 of 1861

selection of test antibodies attached to the array can be used. For example, an antibody array from Full Moon Biosystems can be screened using prostate cancer cell derived vesicles identifying antibodies to Bcl-XL, ERCC1, Keratin 15, CD81/TAPA-1, CD9, Epithelial Specific Antigen (ESA), and Mast Cell Chymase as binding agents, and the proteins identified can be used as biomarkers for the vesicles. The biomarker can be present or absent, underexpressed or overexpressed, mutated, or modified in or on a vesicle and used in characterizing a condition.

[00228] An antibody or synthetic antibody to be used as a binding agent can also be identified through a peptide array. Another method is the use of synthetic antibody generation through antibody phage display. M13 bacteriophage libraries of antibodies (e.g. Fabs) are displayed on the surfaces of phage particles as fusions to a coat protein. Each phage particle displays a unique antibody and also encapsulates a vector that contains the encoding DNA. Highly diverse libraries can be constructed and represented as phage pools, which can be used in antibody selection for binding to immobilized antigens. Antigen-binding phages are retained by the immobilized antigen, and the nonbinding phages are removed by washing. The retained phage pool can be amplified by infection of an *Escherichia coli* host and the amplified pool can be used for additional rounds of selection to eventually obtain a population that is dominated by antigen-binding clones. At this stage, individual phase clones can be isolated and subjected to DNA sequencing to decode the sequences of the displayed antibodies. Through the use of phase display and other methods known in the art, high affinity designer antibodies for vesicles can be generated.

[00229] Bead-based assays can also be used to identify novel binding agents to isolate or detect a vesicle. A test antibody or peptide can be conjugated to a particle. For example, a bead can be conjugated to an antibody or peptide and used to detect and quantify the proteins expressed on the surface of a population of vesicles in order to discover and specifically select for novel antibodies that can target vesicles from specific tissue or tumor types. Any molecule of organic origin can be successfully conjugated to a polystyrene bead through use of a commercially available kit according to manufacturer's instructions. Each bead set can be colored a certain detectable wavelength and each can be linked to a known antibody or peptide which can be used to specifically measure which beads are linked to exosomal proteins matching the epitope of previously conjugated antibodies or peptides. The beads can be dyed with discrete fluorescence intensities such that each bead with a different intensity has a different binding agent as described above.

[00230] For example, a purified vesicle preparation can be diluted in assay buffer to an appropriate concentration according to empirically determined dynamic range of assay. A sufficient volume of coupled beads can be prepared and approximately 1 µĩ of the antibody-coupled beads can be aliqouted into a well and adjusted to a final volume of approximately 50 µĩ. Once the antibody-conjugated beads have been added to a vacuum compatible plate, the beads can be washed to ensure proper binding conditions. An appropriate volume of vesicle preparation can then be added to each well being tested and the mixture incubated, such as for 15-18 hours. A sufficient volume of detection antibodies using detection antibody diluent solution can be prepared and incubated with the mixture for 1 hour or for as long as necessary. The beads can then be washed before the addition of detection antibody (biotin expressing) mixture composed of streptavidin phycoereythin. The beads can then be washed and vacuum aspirated several times before analysis on a suspension array system using software provided with an instrument. The identity of antigens that can be used to selectively extract the vesicles can then be elucidated from the analysis.

PREOMICS EXHIBIT 1002
Page 988 of 1861

[00231] Assays using imaging systems can be used to detect and quantify proteins expressed on the surface of a vesicle in order to discover and specifically select for and enrich vesicles from specific tissue, cell or tumor types. Antibodies, peptides or cells conjugated to multiple well multiplex carbon coated plates can be used. Simultaneous measurement of many analytes in a well can be achieved through the use of capture antibodies arrayed on the patterned carbon working surface. Analytes can then be detected with antibodies labeled with reagents in electrode wells with an enhanced electro-chemiluminescent plate. Any molecule of organic origin can be successfully conjugated to the carbon coated plate. Proteins expressed on the surface of vesicles can be identified from this assay and can be used as targets to specifically select for and enrich vesicles from specific tissue or tumor types.

[00232] The binding agent can also be an aptamer, which refers to nucleic acids that can bond molecules other than their complementary sequence. An aptamer typically contains 30-80 nucleic acids and can have a high affinity towards a certain target molecule (K_d's reported are between 10^{-11}-10^{-6}mole/l). An aptamer for a target can be identified using systematic evolution of ligands by exponential enrichment (SELEX) *{Tuerk & Gold, Science 249:505-510, 1990; Ellington & Szostak, Nature 346:818-822, 1990),* such as described in U.S. Pat. Nos. 5,270,163, 6,482, 594, 6,291, 184, 6,376, 190 and US 6,458, 539. A library of nucleic acids can be contacted with a target vesicle, and those nucleic acids specifically bound to the target are partitioned from the remainder of nucleic acids in the library which do not specifically bind the target. The partitioned nucleic acids are amplified to yield a ligand-enriched pool. Multiple cycles of binding, partitioning, and amplifying (i.e., selection) result in identification of one or more aptamers with the desired activity. Another method for identifying an aptamer to isolate vesicles is described in U.S. Pat. No. 6,376, 190, which describes increasing or decreasing frequency of nucleic acids in a library by their binding to a chemically synthesized peptide. Modified methods, such as Laser SELEX or deSELEX as described in U.S. Patent Publication No. 20090264508 can also be used.

[00233] The term "specific" as used herein in regards to a binding agent can mean that an agent has a greater affinity for its target than other targets, typically with a much great affinity, but does not require that the binding agent is absolutely specific for its target.

Microfluidics

[00234] The methods for isolating or identifying vesicles can be used in combination with microfluidic devices. The methods of isolating or detecting a vesicle, such as described herien, can be performed using a microfluidic device. Microfluidic devices, which may also be referred to as "lab-on-a-chip" systems, biomedical micro-electro-mechanical systems (bioMEMs), or multicomponent integrated systems, can be used for isolating and analyzing a vesicle. Such systems miniaturize and compartmentalize processes that allow for binding of vesicles, detection of biosignatures, and other processes.

[00235] A microfluidic device can also be used for isolation of a vesicle through size differential or affinity selection. For example, a microfluidic device can use one more channels for isolating a vesicle from a biological sample based on size or by using one or more binding agents for isolating a vesicle from a biological sample. A biological sample can be introduced into one or more microfluidic channels, which selectively allows the passage of a vesicle. The selection can be based on a property of the vesicle, such as the size, shape, deformability, or biosignature of the vesicle.

PREOMICS EXHIBIT 1002
Page 989 of 1861

[00236] In one embodiment, a heterogeneous population of vesicles can be introduced into a microfluidic device, and one or more different homogeneous populations of vesicles can be obtained. For example, different channels can have different size selections or binding agents to select for different vesicle populations. Thus, a microfluidic device can isolate a plurality of vesicles wherein at least a subset of the plurality of vesicles comprises a different biosignature from another subset of the plurality of vesicles. For example, the microfluidic device can isolate at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different subsets of vesicles, wherein each subset of vesicles comprises a different biosignature.

[00237] In some embodiments, the microfluidic device can comprise one or more channels that permit further enrichment or selection of a vesicle. A population of vesicles that has been enriched after passage through a first channel can be introduced into a second channel, which allows the passage of the desired vesicle or vesicle population to be further enriched, such as through one or more binding agents present in the second channel.

[00238] Array-based assays and bead-based assays can be used with microfluidic device. For example, the binding agent can be coupled to beads and the binding reaction between the beads and vesicle can be performed in a microfluidic device. Multiplexing can also be performed using a microfluidic device. Different compartments can comprise different binding agents for different populations of vesicles, where each population is of a different cell-of-origin specific vesicle population. In one embodiment, each population has a different biosignature. The hybridization reaction between the microsphere and vesicle can be performed in a microfluidic device and the reaction mixture can be delivered to a detection device. The detection device, such as a dual or multiple laser detection system can be part of the microfluidic system and can use a laser to identify each bead or microsphere by its color-coding, and another laser can detect the hybridization signal associated with each bead.

[00239] Any appropriate microfluidic device can be used in the methods of the invention. Examples of microfluidic devices that may be used, or adapted for use with vesicles, include but are not limited to those described in U.S. Pat. Nos. 7,591,936, 7,581,429, 7,579,136, 7,575,722, 7,568,399, 7,552,741, 7,544,506, 7,541,578, 7,518,726, 7,488,596, 7,485,214, 7,467,928, 7,452,713, 7,452,509, 7,449,096, 7,431,887, 7,422,725, 7,422,669, 7,419,822, 7,419,639, 7,413,709, 7,411,184, 7,402,229, 7,390,463, 7,381,471, 7,357,864, 7,351,592, 7,351,380, 7,338,637, 7,329,391, 7,323,140, 7,261,824, 7,258,837, 7,253,003, 7,238,324, 7,238,255, 7,233,865, 7,229,538, 7,201,881, 7,195,986, 7,189,581, 7,189,580, 7,189,368, 7,141,978, 7,138,062, 7,135,147, 7,125,711, 7,118,910, 7,118,661, 7,640,947, 7,666,361, 7,704,735; and International Patent Publication WO 2010/072410; each of which patents or applications are incorporated herein by reference in their entirety. Another example for use with methods disclosed herein is described in *Chen et al., "Microfluidic isolation and transcriptome analysis of serum vesicles," Lab on a Chip, Dec. 8, 2009 DOI: 10.1039/b916199f.*

[00240] Other microfluidic devices for use with the invention include devices comprising elastomeric layers, valves and pumps, including without limitation those disclosed in U.S. Patent Nos. 5,376,252, 6,408,878, 6,645,432, 6,719,868, 6,793,753, 6,899,137, 6,929,030, 7,040,338, 7,118,910, 7,144,616, 7,216,671, 7,250,128, 7,494,555, 7,501,245, 7,601,270, 7,691,333, 7,754,010, 7,837,946; U.S. Patent Application Nos. 2003/0061687, 2005/0084421, 2005/0112882, 2005/0129581, 2005/0145496, 2005/0201901, 2005/0214173, 2005/0252773, 2006/0006067; and EP Patent Nos. 0527905 and 1065378; each of which application is herein incorporated by reference. In some instances, much or all of the devices are composed of elastomeric material. Certain devices are designed to conduct thermal cycling reactions (e.g., PCR) with devices that include one or more elastomeric

PREOMICS EXHIBIT 1002
Page 990 of 1861

valves to regulate solution flow through the device. The devices can comprise arrays of reaction sites thereby allowing a plurality of reactions to be performed. Thus, the devices can be used to assess circulating microRNAs in a multiplex fashion, including microRNAs isolated from vesicles. In an embodiment, the microfluidic device comprises (a) a first plurality of flow channels formed in an elastomeric substrate; (b) a second plurality of flow channels formed in the elastomeric substrate that intersect the first plurality of flow channels to define an array of reaction sites, each reaction site located at an intersection of one of the first and second flow channels; (c) a plurality of isolation valves disposed along the first and second plurality of flow channels and spaced between the reaction sites that can be actuated to isolate a solution within each of the reaction sites from solutions at other reaction sites, wherein the isolation valves comprise one or more control channels that each overlay and intersect one or more of the flow channels; and (d) means for simultaneously actuating the valves for isolating the reaction sites from each other. Various modifications to the basic structure of the device are envisioned within the scope of the invention. MicroRNAs can be detected in each of the reaction sites by using PCR methods. For example, the method can comprise the steps of the steps of: (i) providing a microfluidic device, the microfluidic device comprising: a first fluidic channel having a first end and a second end in fluid communication with each other through the channel; a plurality of flow channels, each flow channel terminating at a terminal wall; wherein each flow channel branches from and is in fluid communication with the first fluidic channel, wherein an aqueous fluid that enters one of the flow channels from the first fluidic channel can flow out of the flow channel only through the first fluidic channel; and, an inlet in fluid communication with the first fluidic channel, the inlet for introducing a sample fluid; wherein each flow channel is associated with a valve that when closed isolates one end of the flow channel from the first fluidic channel, whereby an isolated reaction site is formed between the valve and the terminal wall; a control channel; wherein each the valve is a deflectable membrane which is deflected into the flow channel associated with the valve when an actuating force is applied to the control channel, thereby closing the valve; and wherein when the actuating force is applied to the control channel a valve in each of the flow channels is closed, so as to produce the isolated reaction site in each flow channel; (ii) introducing the sample fluid into the inlet, the sample fluid filling the flow channels; (iii) actuating the valve to separate the sample fluid into the separate portions within the flow channels; (iv) amplifying the nucleic acid in the sample fluid; (v) analyzing the portions of the sample fluid to determine whether the amplifying produced the reaction. The sample fluid can contain an amplifiable nucleic acid target, e.g., a microRNA, and the conditions can be polymerase chain reaction (PCR) conditions, so that the reaction results in a PCR product being formed.

[00241] In an embodiment, the PCR used to detect microRNA is digital PCR, which is described by Brown, et al., U.S. Pat. No. 6,143,496, titled "Method of sampling, amplifying and quantifying segment of nucleic acid, polymerase chain reaction assembly having nanoliter-sized chambers and methods of filling chambers", and by Vogelstein, et al, U.S. Pat. No. 6,446,706, titled "Digital PCR", both of which are hereby incorporated by reference in their entirety. In digital PCR, a sample is partitioned so that individual nucleic acid molecules within the sample are localized and concentrated within many separate regions, such as the reaction sites of the microfluidic device described above. The partitioning of the sample allows one to count the molecules by estimating according to Poisson. As a result, each part will contain "0" or "1" molecules, or a negative or positive reaction, respectively. After PCR amplification, nucleic acids may be quantified by counting the regions that contain PCR end-product, positive reactions. In conventional PCR, starting copy number is proportional to

PREOMICS EXHIBIT 1002
Page 991 of 1861

the number of PCR amplification cycles. Digital PCR, however, is not dependent on the number of amplification cycles to determine the initial sample amount, eliminating the reliance on uncertain exponential data to quantify target nucleic acids and providing absolute quantification. Thus, the method can provide a sensitive approach to detecting microRNAs in a sample.

[00242] In one embodiment, a microfluidic device for isolating or detecting a vesicle comprises a channel of less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, of 60 mm in width, or between about 2-60, 3-50, 3-40, 3-30, 3-20, or 4-20 mm in width. The microchannel can have a depth of less than about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65 or 70 μτη, or between about 10-70, 10-40, 15-35, or 20-30 μπι. Furthermore, the microchannel can have a length of less than about 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 cm. The microfluidic device can have grooves on its ceiling that are less than about 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 6, 65, 70, 75, or 80 μτη wide, or between about 40-80, 40-70, 40-60 or 45-55 μτη wide. The grooves can be less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 μτη deep, such as between about 1-50, 5-40, 5-30, 3-20 or 5-15 μπι.

[00243] The microfluidic device can have one or more binding agents attached to a surface in a channel, or present in a channel. For example, the microchannel can have one or more capture agents, such as a capture agent for EpCam, CD9, PCSA, CD63, CD81, PSMA, B7H3, PSCA, ICAM, STEAP, and EGFR. In one embodiment, a microchannel surface is treated with avidin and a capture agent, such as an antibody, that is biotinylated can be injected into the channel to bind the avidin. In other embodiments, the capture agents are present in chambers or other components of a microfluidic device. The capture agents can also be attached to beads that can be manipulated to move through the microfluidic channels. In one embodiment, the capture agents are attached to magnetic beads. The beads can be manipulated using magnets.

[00244] A biological sample can be flowed into the microfluidic device, or a microchannel, at rates such as at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 μi per minute, such as between about 1-50, 5-40, 5-30, 3-20 or 5-15 μΐ per minute. One or more vesicles can be captured and directly detected in the microfluidic device. Alternatively, the captured vesicle may be released and exit the microfluidic device prior to analysis. In another embodiment, one or more captured vesicles are lysed in the microchannel and the lysate can be analyzed, e.g., to examine payload within the vesicles. Lysis buffer can be flowed through the channel and lyse the captured vesicles. For example, the lysis buffer can be flowed into the device or microchannel at rates such as at least about a, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 26, 27, 28, 29, 30, 35, 40, 45, or 50 μΐ per minute, such as between about 1-50, 5-40, 10-30, 5-30 or 10-35 μΐ per minute. The lysate can be collected and analyzed, such as performing RT-PCR, PCR, mass spectrometry, Western blotting, or other assays, to detect one or more biomarkers of the vesicle.

[00245] The various isolation and detection systems described herein can be used to isolate or detect circulating biomarkers such as vesicles that are informative for diagnosis, prognosis, disease stratification, theranosis, prediction of responder / non-responder status, disease monitoring, treatment monitoring and the like as related to such diseases and disorders. Combinations of the isolation techniques are within the scope of the invention. In a non-limiting example, a sample can be run through a chromatography column to isolate vesicles based on a

PREOMICS EXHIBIT 1002
Page 992 of 1861

property such as size of electrophoretic motility, and the vesicles can then be passed through a microfluidic device. Binding agents can be used before, during or after these steps.

Cell and Disease-Specific Vesicles

[00246] The bindings agent disclosed herein can be used to isolate or detect a vesicle, such as a cell-of-origin vesicle or vesicle with a specific biosignature. The binding agent can be used to isolate or detect a heterogeneous population of vesicles from a sample or can be used to isolate or detect a homogeneous population of vesicles, such as cell-of-origin specific vesicles with specific biosignatures, from a heterogeneous population of vesicles.

[00247] A homogeneous population of vesicles, such as cell-of-origin specific vesicles, can be analyzed and used to characterize a phenotype for a subject. Cell-of-origin specific vesicles are esicles derived from specific cell types, which can include, but are not limited to, cells of a specific tissue, cells from a specific tumor of interest or a diseased tissue of interest, circulating tumor cells, or cells of maternal or fetal origin. The vesicles may be derived from tumor cells or lung, pancreas, stomach, intestine, bladder, kidney, ovary, testis, skin, colorectal, breast, prostate, brain, esophagus, liver, placenta, or fetal cells. The isolated vesicle can also be from a particular sample type, such as urinary vesicle.

[00248] A cell-of-origin specific vesicle from a biological sample can be isolated using one or more binding agents that are specific to a cell-of-origin. Vesicles for analysis of a disease or condition can be isolated using one or more binding agent specific for biomarkers for that disease or condition.

[00249] A vesicle can be concentrated prior to isolation or detection of a cell-of-origin specific vesicle, such as through centrifugation, chromatography, or filtration, as described above, to produce a heterogeneous population of vesicles prior to isolation of cell-of-origin specific vesicles. Alternatively, the vesicle is not concentrated, or the biological sample is not enriched for a vesicle, prior to isolation of a cell-of-origin vesicle.

[00250] **FIG. 1B** illustrates a flowchart which depicts one method **6100B** for isolating or identifying a cell-of-origin specific vesicle. First, a biological sample is obtained from a subject in step **6102.** The sample can be obtained from a third party or from the same party performing the analysis. Next, cell-of-origin specific vesicles are isolated from the biological sample in step **6104.** The isolated cell-of-origin specific vesicles are then analyzed in step **6106** and a biomarker or biosignature for a particular phenotype is identified in step **6108.** The method may be used for a number of phenotypes. In some embodiments, prior to step **6104,** vesicles are concentrated or isolated from a biological sample to produce a homogeneous population of vesicles. For example, a heterogeneous population of vesicles may be isolated using centrifugation, chromatography, filtration, or other methods as described above, prior to use of one or more binding agents specific for isolating or identifying vesicles derived from specific cell types.

[00251] A cell-of-origin specific vesicle can be isolated from a biological sample of a subject by employing one or more binding agents that bind with high specificity to the cell-of-origin specific vesicle. In some instances, a single binding agent can be employed to isolate a cell-of-origin specific vesicle. In other instances, a combination of binding agents may be employed to isolate a cell-of-origin specific vesicle. For example, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75, or 100 different binding agents may be used to isolate a cell-of-origin vesicle. Therefore, a vesicle population (e.g., vesicles having the same binding agent profile) can be identified by using a single or a plurality of binding agents.

PREOMICS EXHIBIT 1002
Page 993 of 1861

[00252] One or more binding agents can be selected based on their specificity for a target antigen(s) that is specific to a cell-of-origin, e.g., a cell-of-origin that is related to a tumor, autoimmune disease, cardiovascular disease, neurological disease, infection or other disease or disorder. The cell-of-origin can be from a cell that is informative for a diagnosis, prognosis, disease stratification, theranosis, prediction of responder / non-responder status, disease monitoring, treatment monitoring and the like as related to such diseases and disorders. The cell-of-origin can also be from a cell useful to discover biomarkers for use thereto. Non-limiting examples of antigens which may be used singularly, or in combination, to isolate a cell-of-origin specific vesicle, disease specific vesicle, or tumor specific vesicle, are shown in **Fig. 1** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein, and are also described herein. The antigen can comprise membrane bound antigens which are accessible to binding agents. The antigen can be a biomarker related to characterizing a phenotype.

[00253] One of skill will appreciate that any applicable antigen that can be used to isolate an informative vesicle is contemplated by the invention. Binding agents, e.g., antibodies, aptamers and lectins, can be chosen that recognize surface antigens and/or fragments thereof, as outlined herein. The binding agents can recognize antigens specific to the desired cell type or location and/or recognize biomarkers associated with the desired cells. The cells can be, e.g., tumor cells, other diseased cells, cells that serve as markers of disease such as activated immune cells, etc. One of skill will appreciate that binding agents for any cells of interest can be useful for isolating vesicles associated with those cells. One of skill will further appreciate that the binding agents disclosed herein can be used for detecting vesicles of interest. As a non-limiting example, a binding agent to a vesicle biomarker can be labeled directly or indirectly in order to detect vesicles bound by one of more of the same or different binding agents.

[00254] A number of targets for binding agents useful for binding to vesicles associated with cancer, autoimmune diseases, cardiovascular diseases, neurological diseases, infection or other disease or disorders are presented in **Table 4.** A vesicle derived from a cell associated with one of the listed disorders can be characterized using one of the antigens in the table. The binding agent, e.g., an antibody or aptamer, can recognize an epitope of the listed antigens, a fragment thereof, or binding agents can be used against any appropriate combination. Other antigens associated with the disease or disorder can be recognized as well in order to characterize the vesicle. One of skill will appreciate that any applicable antigen that can be used to assess an informative vesicle is contemplated by the invention for isolation, capture or detection in order to characterize a vesicle.

Table 4: Illustrative Antigens for Use in Characterizing Various Diseases and Disorders

Disease or disorder	Target
Breast cancer, e.g., glandular or stromal cells	BCA-225, hsp70, MART1, ER, VEGFA, Class III b-tubulin, HER2/neu (for Her2+ breast cancer), GPR30, ErbB4 (JM) isoform, MPR8, MISIIR
Breast cancer	CD9, MIS Rii, ER, CD63, MUC1, HER3, STAT3, VEGFA, BCA, CA125, CD24, EPCAM, ERB B4
Breast cancer	BCA-225, hsp70, MART1, ER, VEGFA, Class III b-tubulin, HER2/neu (e.g., for Her2+ breast cancer), GPR30, ErbB4 (JM) isoform, MPR8, MISIIR, CD9, EphA2, EGFR, B7H3, PSM, PCSA, CD63, STEAP, CD81, ICAM1, A33, DR3, CD66e, MFG-E8, TROP-2, Mammaglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4,

PREOMICS EXHIBIT 1002
Page 994 of 1861

	NGAL, EpCam, neurokinin receptor-1 (NK-1 or NK-1R), NK-2, Pai-1, CD45, CD10, HER2/ERBB2, AGTR1, NPY1R, MUC1, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUC1 secreted), CA27.29 (MUC1 secreted), NMDAR1, NMDAR2, MAGEA, CTAG1B, NY-ESO-1, SPB, SPC, NSE, PGP9.5, a progesterone receptor (PR) or its isoform (PR(A) or PR(B)), P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, IC3b, mesothelin, SPA, AQP5, GPCR, hCEA-CAM, PTP IA-2, CABYR, TMEM211, ADAM28, UNC93A, MUC17, MUC2, IL10R-beta, BCMA, HVEM/TNFRSF14, Trappin-2 Elafin, ST2/IL1 R4, TNFRF14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNF
Breast cancer	CD10, NPGP/NPFF2, HER2/ERBB2, AGTR1, NPY1R, neurokinin receptor-1 (NK-1 or NK-1R), NK-2, MUC1, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUC1 secreted), CA27.29 (MUC1 secreted), NMDAR1, NMDAR2, MAGEA, CTAG1B, NY-ESO-1
Breast cancer	SPB, SPC, NSE, PGP9.5, CD9, P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, EGFR, B7H3, IC3b, MUC1, mesothelin, SPA, PCSA, CD63, STEAP, AQP5, CD81, DR3, PSM, GPCR, EphA2, hCEA-CAM, PTP IA-2, CABYR, TMEM211, ADAM28, UNC93A, A33, CD24, CD10, NGAL, EpCam, MUC17, TROP-2, MUC2, IL10R-beta, BCMA, HVEM/TNFRSF14, Trappin-2 Elafin, ST2/IL1 R4, TNFRF14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNFR
Breast cancer	BRCA, MUC-1, MUC 16, CD24, ErbB4, ErbB2 (HER2), ErbB3, HSP70, Mammaglobin, PR, PR(B), VEGFA
Ovarian Cancer	CA125, VEGFR2, HER2, MISIIR, VEGFA, CD24, c-reactive protein EGFR, EGFRvIII, apolipoprotein AI, apolipoprotein CIII, myoglobin, tenascin C, MSH6, claudin-3, claudin-4, caveolin-1, coagulation factor III, CD9, CD36, CD37, CD53, CD63, CD81, CD136, CD147, Hsp70, Hsp90, Rab13, Desmocollin-1, EMP-2, CK7, CK20, GCDF15, CD82, Rab-5b, Annexin V, MFG-E8, HLA-DR, CD95
Lung Cancer	CYFRA21-1, TPA-M, TPS, CEA, SCC-Ag, XAGE-1b, HLA Class 1, TA-MUC1, KRAS, hENT1, kinin B1 receptor, kinin B2 receptor, TSC403, HTI56, DC-LAMP
Lung Cancer	SPB, SPC, PSP9.5, NDUFB7, gal3-b2c10, iC3b, MUC1, GPCR, CABYR and muc17
Colorectal Cancer	CEA, MUC2, GPA33, CEACAM5, ENFB1, CCSA-3, CCSA-4, ADAM10, CD44, NG2, ephrin B1, plakoglobin, galectin 4, RACK1, tetraspanin-8, FASL, A33, CEA, EGFR, dipeptidase 1, PTEN, Na(+)-dependent glucose transporter, UDP-glucuronosyltransferase 1A, TMEM211, CD24
Prostate Cancer	PSA, TMPRSS2, FASLG, TNFSF10, PSMA, NGEP, Il-7RI, CSCR4, CysLT1R, TRPM8, Kv1.3, TRPV6, TRPM8, PSGR, MISIIR, galectin-3, PCA3, TMPRSS2:ERG
Brain Cancer	PRMT8, BDNF, EGFR, DPPX, Elk, Densin-180, BAI2, BAI3
Blood Cancer (hematological malignancy)	CD44, CD58, CD31, CD11a, CD49d, GARP, BTS,

PREOMICS EXHIBIT 1002
Page 995 of 1861

	Raftlin
Melanoma	DUSP1, TYRP1, SILV, MLANA, MCAM, CD63, Alix, hsp70, meosin, p120 catenin, PGRL, syntaxin binding protein 1 & 2, caveolin
Liver Cancer (hepatocellular carcinoma)	HBxAg, HBsAg, NLT
Cervical Cancer	MCT-1, MCT-2, MCT-4
Endometrial Cancer	Alpha V Beta 6 integrin
Psoriasis	flt-1, VPF receptors, kdr
Autoimmune Disease	Tim-2
Irritable Bowel Disease (IBD or Syndrome (IBS)	IL-16, IL-1beta, IL-12, TNF-alpha, interferon-gamma, IL-6, Rantes, II-12, MCP-1, 5HT
Diabetes, e.g., pancreatic cells	IL-6, CRP, RBP4
Barrett's Esophagus	p53, MUC1, MUC6
Fibromyalgia	neopterin, gp130
Benign Prostatic Hyperplasia (BPH)	KIA1, intact fibronectin
Multiple Sclerosis	B7, B7-2, CD-95 (fas), Apo-1/Fas
Parkinson's Disease	PARK2, ceruloplasmin, VDBP, tau, DJ-1
Rheumatic Disease	Citrulinated fibrin a-chain, CD5 antigen-like fibrinogen fragment D, CD5 antigen-like fibrinogen fragment B, TNF alpha
Alzheimer's Disease	APP695, APP751 or APP770, BACE1, cystatin C, amyloid β, T-tau, complement factor H, alpha-2-macroglobulin
Head and Neck Cancer	EGFR, EphB4, Ephrin B2
Gastrointestinal Stromal Tumor (GIST)	c-kit PDGFRA, NHE-3
Renal Cell Carcinoma	c PDGFRA, VEGF, HIF 1 alpha
Schizophrenia	ATP5B, ATP5H, ATP6V1B, DNM1
Peripheral Neuropathic Pain	OX42, ED9
Chronic Neuropathic Pain	chemokine receptor (CCR2/4)
Prion Disease	PrPSc, 14-3-3 zeta, S-100, AQP4
Stroke	S-100, neuron specific enolase, PARK7, NDKA, ApoC-I, ApoC-III, SAA or AT-III fragment, Lp-PLA2, hs-CRP
Cardiovascular Disease	FATP6
Esophageal Cancer	CaSR
Tuberculosis	antigen 60, HSP, Lipoarabinomannan, Sulfolipid, antigen of acylated trehalose family, DAT, TAT, Trehalose 6,6 – dimycolate (cord-factor) antigen
HIV	gp41, gp120
Autism	VIP, PACAP, CGRP, NT3
Asthma	YKL-40, S-nitrosothiols, SSCA2, PAI, amphiregulin, periostin
Lupus	TNFR
Cirrhosis	NLT, HBsAg
Influenza	hemagglutinin, neurominidase
Vulnerable Plaque	Alpha v. Beta 3 integrin, MMP9

[00255] The foregoing **Table 4,** as well as other biomarker lists disclosed here are illustrative, and Applicants contemplate incorporating various biomarkers disclosed across different disease states or conditions. For example, method of the invention may use various biomarkers across different diseases or conditions, where the biomarkers are useful for providing a diagnostic, prognostic or theranostic signature. In one embodiment, angiogenic, inflammatory or immune-associated antigens (or biomarkers) disclosed herein or know in the art can be used in methods of the invention to screen a biological sample in identification of a biosignature. Indeed, the flexibility of Applicants' multiplex approach to assessing microvesicle populations facilitates assessing various markers (and in some instances overlapping markers) for different conditions or diseases whose etiology

PREOMICS EXHIBIT 1002
Page 996 of 1861

necessarily may share certain cellular and biological mechanisms, e.g., different cancers implicating biomarkers for angiogenesis, or immune response regulation or modulation. The combination of such overlapping biomarkers with tissue or cell-specific biomarkers, along with microvesicle-associated biomarkers provides a powerful series of tools for practicing the methods and compositions of the invention.

[00256] A cell-of-origin specific vesicle may be isolated using novel binding agents, using methods as described herein. Furthermore, a cell-of-origin specific vesicle can also be isolated from a biological sample using isolation methods based on cellular binding partners or binding agents of such vesicles. Such cellular binding partners can include but are not limited to peptides, proteins, RNA, DNA, apatmers, cells or serum-associated proteins that only bind to such vesicles when one or more specific biomarkers are present. Isolation or deteciton of a cell-of-origin specific vesicle can be carried out with a single binding partner or binding agent, or a combination of binding partners or binding agents whose singular application or combined application results in cell-of-origin specific isolation or detection. Non-limiting examples of such binding agents are provided in **Fig. 2** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein. For example, a vesicle for characterizing breast cancer can be isolated with one or more binding agents including, but not limited to, estrogen, progesterone, trastuzumab, CCND1, MYC PNA, IGF-1 PNA, MYC PNA, SC4 aptamer (Ku), AII-7 aptamer (ERB2), Galectin -3, mucin-type O-glycans, L-PHA, Galectin-9, or any combination thereof.

[00257] A binding agent may also be used for isolating or detecting a cell-of-origin specific vesicle based on: i) the presence of antigens specific for cell-of-origin specific vesicles; ii) the absence of markers specific for cell-of-origin specific vesicles; or iii) expression levels of biomarkers specific for cell-of-origin specific vesicles. A heterogeneous population of vesicles can be applied to a surface coated with specific binding agents designed to rule out or identify the cell-of-origin characteristics of the vesicles. Various binding agents, such as antibodies, can be arrayed on a solid surface or substrate and the heterogeneous population of vesicles is allowed to contact the solid surface or substrate for a sufficient time to allow interactions to take place. Specific binding or non-binding to given antibody locations on the array surface or substrate can then serve to identify antigen specific characteristics of the vesicle population that are specific to a given cell-of-origin. That is, binding events can signal the presence of a vesicle having an antigen recognized by the bound antibody. Conversely, lack of binding events can signal the absence of vesicles having an antigen recognized by the bound antibody.

[00258] A cell-of-origin specific vesicle can be enriched or isolated using one or more binding agents using a magnetic capture method, fluorescence activated cell sorting (FACS) or laser cytometry as described above. Magnetic capture methods can include, but are not limited to, the use of magnetically activated cell sorter (MACS) microbeads or magnetic columns. Examples of immunoaffinity and magnetic particle methods that can be used are described in U.S. Patent Nos. 4,55 1,435, 4,795,698, 4,925,788, 5,108,933, 5,186,827, 5,200,084 or 5,158,871 . A cell-of-origin specific vesicle can also be isolated following the general methods described in U.S. Patent No. 7,399,632, by using combination of antigens specific to a vesicle.

[00259] Any other appropriate method for isolating or otherwise enriching the cell-of-origin specific vesicles with respect to a biological sample may also be used in combination with the present invention. For example, size exclusion chromatography such as gel permeation columns, centrifugation or density gradient centrifugation, and filtration methods can be used in combination with the antigen selection methods described

PREOMICS EXHIBIT 1002
Page 997 of 1861

herein. The cell-of-origin specific vesicles may also be isolated following the methods described in *Koga et al., Anticancer Research, 25:3703-3708 (2005), Taylor et al, Gynecologic Oncology, 110:13-21 (2008), Nanjee et al, Clin Chem, 2000;46:207-223* or U.S Patent No. 7,232,653.

[00260] Vesicles can be isolated and/or detected to provide diagnosis, prognosis, disease stratification, theranosis, prediction of responder / non-responder status, disease monitoring, treatment monitoring and the like. In one embodiment, vesicles are isolated from cells having a disease or disorder, e.g., cells derived from a tumor or malignant growth, a site of autoimmune disease, cardiovascular disease, neurological disease, or infection. In some embodiments, the isolated vesicles are derived from cells related to such diseases and disorders, e.g., immune cells that play a role in the etiology of the disease and whose analysis is informative for a diagnosis, prognosis, disease stratification, theranosis, prediction of responder / non-responder status, disease monitoring, treatment monitoring and the like as relates to such diseases and disorders. The vesicles are further useful to discover novel biomarkers. By identifying biomarkers associated with vesicles, isolated vesicles can be assessed for characterizing a phenotype as described herein.

[00261] In some embodiments, methods of the invention are directed to characterizing presence of a cancer or likelihood of a cancer occurring in an individual by assessing one or more microvesicle population present in a biological sample from an individual. Microvesicles can be isolated using one or more processes disclosed herein or practiced in the art.

[00262] Such microvesicles populations can each separately or collectively provide a disease phenotype characterization for the individual by comparing the biomarker profile, or biosignature, for the microvesicle population(s) with a reference sample to provide a diagnostic, prognostic or theranostic characterization for the test sample.

[00263] The vesicle population(s) can be assessed from various biological samples and bodily fluids such as disclosed herein.

Biomarker Assessment

[00264] In an aspect of the invention, a phenotype of a subject is characterized by analyzing a biological sample and determining the presence, level, amount, or concentration of one or more populations of circulating biomarkers in the sample, e.g., circulating vesicles, proteins or nucleic acids. In embodiments, characterization includes determining whether the circulating biomarkers in the sample are altered as compared to a reference, which can also be referred to a standard or a control. An alteration can include any measurable difference between the sample and the reference, including without limitation an absolute presence or absence, a quantitative level, a relative level compared to a reference, e.g., the level of all vesicles present, the level of a housekeeping marker, and/or the level of a spiked-in marker, an elevated level, a decreased level, overexpression, underexpression, differential expression, a mutation or other altered sequence, a modification (glycosylation, phosphorylation, epigenetic change) and the like. In some embodiments, circulating biomarkers are purified or concentrated from a sample prior to determining their amount. Unless otherwise specified, "purified" or "isolated" as used herein refer to partial or complete purification or isolation. In other embodiments, circulating biomarkers are directly assessed from a sample, without prior purification or concentration. Circulating vesicles can be cell-of-origin specific vesicles or vesicles with a specific biosignature. A biosignature includes specific pattern of biomarkers, e.g., patterns of biomarkers indicative of a phenotype that is desireable to detect, such as a disease phenotype. The biosignature can comprise one or more circulating

PREOMICS EXHIBIT 1002
Page 998 of 1861

biomarkers. A biosignature can be used when characterizing a phenotype, such as a diagnosis, prognosis, theranosis, or prediction of responder / non-responder status. In some embodiments, the biosignature is used to determine a physiological or biological state, such as pregnancy or the stage of pregnancy. The biosignature can also be used to determine treatment efficacy, stage of a disease or condition, or progression of a disease or condition. For example, the amount of one or more vesicles can be proportional or inversely proportional to an increase in disease stage or progression. The detected amount of vesicles can also be used to monitor progression of a disease or condition or to monitor a subject's response to a treatment.

[00265] The circulating biomarkers can be evaluated by comparing the level of circulating biomarkers with a reference level or value. The reference value can be particular to physical or temporal endpoint. For example, the reference value can be from the same subject from whom a sample is assessed, or the reference value can be from a representative population of samples (e.g., samples from normal subjects not exhibiting a symptom of disease). Therefore, a reference value can provide a threshold measurement which is compared to a subject sample's readout for a biosignature assayed in a given sample. Such reference values may be set according to data pooled from groups of sample corresponding to a particular cohort, including but not limited to age (e.g., newborns, infants, adolescents, young, middle-aged adults, seniors and adults of varied ages), racial/ethnic groups, normal versus diseased subjects, smoker v. non-smoker, subject receiving therapy versus untreated subject, different time points of treatment for a particular individual or group of subjects similarly diagnosed or treated or combinations thereof. Furthermore, by determining a biosignature at different timepoints of treatment for a particular individual, the individual's response to the treatment or progression of a disease or condition for which the individual is being treated for, can be monitored.

[00266] A reference value may be based on samples assessed from the same subject so to provide individualized tracking. In some embodiments, frequent testing of a biosignature in samples from a subject provides better comparisons to the reference values previously established for that subject. Such time course measurements are used to allow a physician to more accurately assess the subject's disease stage or progression and therefore inform a better decision for treatment. In some cases, the variance of a biosignature is reduced when comparing a subject's own biosignature over time, thus allowing an individualized threshold to be defined for the subject, e.g., a threshold at which a diagnosis is made. Temporal intrasubject variation allows each individual to serve as their own longitudinal control for optimum analysis of disease or physiological state. As an illustrative example, consider that the level of vesicles derived from prostate cells is measured in a subject's blood over time. A spike in the level of prostate-derived vesicles in the subject's blood can indicate hyperproliferation of prostate cells, e.g., due to prostate cancer.

[00267] Reference values can be established for unaffected individuals (of varying ages, ethnic backgrounds and sexes) without a particular phenotype by determining the biosignature of interest in an unaffected individual. For example, a reference value for a reference population can be used as a baseline for detection of one or more circulating biomarker populations in a test subject. If a sample from a subject has a level or value that is similar to the reference, the subject can be identified to not have the disease, or of having a low likelihood of developing a disease.

[00268] Alternatively, reference values or levels can be established for individuals with a particular phenotype by determining the amount of one or more populations of vesicles in an individual with the phenotype. In addition, an index of values can be generated for a particular phenotype. For example, different disease stages

PREOMICS EXHIBIT 1002
Page 999 of 1861

can have different values, such as obtained from individuals with the different disease stages. A subject's value can be compared to the index and a diagnosis or prognosis of the disease can be determined, such as the disease stage or progression wherein the subject's levels most closely correlate with the index. In other embodiments, an index of values is generated for therapeutic efficacies. For example, the level of vesicles of individuals with a particular disease can be generated and noted what treatments were effective for the individual. The levels can be used to generate values of which is a subject's value is compared, and a treatment or therapy can be selected for the individual, e.g., by predicting from the levels whether the subject is likely to be a responder or non-responder for a treatment.

[00269] In some embodiments, a reference value is determined for individuals unaffected with a particular cancer, by isolating or detecting circulating biomarkers with an antigen that specifically targets biomarkers for the particular cancer. As a non-limiting example, individuals with varying stages of colorectal cancer and noncancerous polyps can be surveyed using the same techniques described for unaffected individuals and the levels of circulating vesicles for each group can be determined. In some embodiments, the levels are defined as means ± standard deviations from at least two separate experiments, performed in at least duplicate or triplicate. Comparisons between these groups can be made using statistical tests to determine statistical significance of distinguishing biomarkers observed. In some embodiments, statistical significance is determined using a parametric statistical test. The parametric statistical test can comprise, without limitation, a fractional factorial design, analysis of variance (ANOVA), a t-test, least squares, a Pearson correlation, simple linear regression, nonlinear regression, multiple linear regression, or multiple nonlinear regression. Alternatively, the parametric statistical test can comprise a one-way analysis of variance, two-way analysis of variance, or repeated measures analysis of variance. In other embodiments, statistical significance is determined using a nonparametric statistical test. Examples include, but are not limited to, a Wilcoxon signed-rank test, a Mann-Whitney test, a Kruskal-Wallis test, a Friedman test, a Spearman ranked order correlation coefficient, a Kendall Tau analysis, and a nonparametric regression test. In some embodiments, statistical significance is determined at a p-value of less than 0.05, 0.01, 0.005, 0.001, 0.0005, or 0.0001 . The p-values can also be corrected for multiple comparisons, e.g., using a Bonferroni correction, a modification thereof, or other technique known to those in the art, e.g., the Hochberg correction, Holm-Bonferroni correction, Šidak correction, Dunnett's correction or Tukey's multiple comparisons. In some embodiments, an ANOVA is followed by Tukey's correction for post-test comparing of the biomarkers from each population. A biosignature comprising more than one marker can be evaluated using multivariate modeling techniques to build a classifier using techniques described herein or known in the art.

[00270] Reference values can also be established for disease recurrence monitoring (or exacerbation phase in MS), for therapeutic response monitoring, or for predicting responder / non-responder status.

[00271] In some embodiments, a reference value for vesicles is determined using an artificial vesicle, also referred to herein as a synthetic vesicle. Methods for manufacturing artificial vesicles are known to those of skill in the art, e.g., using liposomes. Artificial vesicles can be manufactured using methods disclosed in US20060222654 and US4448765, which are incorporated herein by reference in its entirety. Artificial vesicles can be constructed with known markers to facilitate capture and/or detection. In some embodiments, artificial vesicles are spiked into a bodily sample prior to processing. The level of intact synthetic vesicle can be tracked during processing, e.g., using filtration or other isolation methods disclosed herein, to provide a control for the

PREOMICS EXHIBIT 1002
Page 1000 of 1861

amount of vesicles in the initial versus processed sample. Similarly, artificial vesicles can be spiked into a sample before or after any processing steps. In some embodiments, artificial vesicles are used to calibrate equipment used for isolation and detection of vesicles.

[00272] Artificial vesicles can be produced and used a control to test the viability of an assay, such as a bead-based assay. The artificial vesicle can bind to both the beads and to the detection antibodies. Thus, the artificial vesicle contains the amino acid sequence/conformation that each of the antibodies binds. The artificial vesicle can comprise a purified protein or a synthetic peptide sequence to which the antibody binds. The artificial vesicle could be a bead, e.g., a polystyrene bead, that is capable of having biological molecules attached thereto. If the bead has an available carboxyl group, then the protein or peptide could be attached to the bead via an available amine group, such as using carbodiimide coupling.

[00273] In another embodiment, the artificial vesicle can be a polystyrene bead coated with avidin and a biotin is placed on the protein or peptide of choice either at the time of synthesis or via a biotin-maleimide chemistry. The proteins/peptides to be on the bead can be mixed together in ratio specific to the application the artificial vesicle is being used for, and then conjugated to the bead. These artificial vesicles can then serve as a link between the capture beads and the detection antibodies, thereby providing a control to show that the components of the assay are working properly.

[00274] The value can be a quantitative or qualitative value. The value can be a direct measurement of the level of vesicles (example, mass per volume), or an indirect measure, such as the amount of a specific biomarker. The value can be a quantitative, such as a numerical value. In other embodiments, the value is qualitiative, such as no vesicles, low level of vesicles, medium level, high level of vesicles, or variations thereof.

[00275] The reference value can be stored in a database and used as a reference for the diagnosis, prognosis, theranosis, disease stratification, disease monitoring, treatment monitoring or prediction of non-responder / responder status of a disease or condition based on the level or amount of circulating biomarkers, such as total amount of vesicles or microRNA, or the amount of a specific population of vesicles or microRNA, such as cell-of-origin specific vesicles or microRNA or microRNA from vesicles with a specific biosignature. In an illustrative example, consider a method of determining a diagnosis for a cancer. Vesicles or other circulating biomarkers from reference subjects with and without the cancer are assessed and stored in the database. The reference subjects provide biosignature indicative of the cancer or of another state, e.g., a healthy state. A sample from a test subject is then assayed and the microRNA biosignature is compared against those in the database. If the subject's biosignature correlates more closely with reference values indicative of cancer, a diagnosis of cancer may be made. Conversely, if the subject's biosignature correlates more closely with reference values indicative of a healthy state, the subject may be determined to not have the disease. One of skill will appreciate that this example is non-limiting and can be expanded for assessing other phenotypes, e.g., other diseases, prognosis, theranosis, disease stratification, disease monitoring, treatment monitoring or prediction of non-responder / responder status, and the like.

[00276] A biosignature for characterizing a phenotype can be determined by detecting circulating biomarkers such as vesicles, including biomarkers associate with vesicles such as surface antigens or payload. The payload, e.g., protein or species of RNA such as mRNA or microRNA, can be assessed within a vesicle. Alternately, the payload in a sample is analyzed to characterize the phenotype without isolating the payload from the vesicles. Many analytical techniques are available to assess vesicles. In some embodiments, vesicle levels are

PREOMICS EXHIBIT 1002
Page 1001 of 1861

characterized using mass spectrometry, flow cytometry, immunocytochemical staining, Western blotting, electrophoresis, chromatography or x-ray crystallography in accordance with procedures known in the art. For example, vesicles can be characterized and quantitatively measured using flow cytometry as described in *Clayton et al., Journal of Immunological Methods 2001; 163-1 74,* which is herein incorporated by reference in its entirety. Vesicle levels may be determined using binding agents as described above. For example, a binding agent to vesicles can be labeled and the label detected and used to determine the amount of vesicles in a sample. The binding agent can be bound to a substrate, such as arrays or particles, such as described above. Alternatively, the vesicles may be labeled directly.

[00277] Electrophoretic tags or eTags can be used to determine the amount of vesicles. eTags are small fluorescent molecules linked to nucleic acids or antibodies and are designed to bind one specific nucleic acid sequence or protein, respectively. After the eTag binds its target, an enzyme is used to cleave the bound eTag from the target. The signal generated from the released eTag, called a "reporter," is proportional to the amount of target nucleic acid or protein in the sample. The eTag reporters can be identified by capillary electrophoresis. The unique charge-to-mass ratio of each eTag reporter—that is, its electrical charge divided by its molecular weight-makes it show up as a specific peak on the capillary electrophoresis readout Thus by targeting a specific biomarker of a vesicle with an eTag, the amount or level of vesicles can be determined.

[00278] The vesicle level can determined from a heterogeneous population of vesicles, such as the total population of vesicles in a sample. Alternatively, the vesicles level is determined from a homogenous population, or substantially homogenous population of vesicles, such as the level of specific cell-of-origin vesicles, such as vesicles from prostate cancer cells. In yet other embodiments, the level is determined for vesicles with a particular biomarker or combination of biomarkers, such as a biomarker specific for prostate cancer. Determining the level vesicles can be performed in conjunction with determining the biomarker or combination of biomarkers of a vesicle. Alternatively, determining the amount of vesicle may be performed prior to or subsequent to determining the biomarker or combination of biomarkers of the vesicles.

[00279] Determining the amount of vesicles can be assayed in a multiplexed manner. For example, determining the amount of more than one population of vesicles, such as different cell-of-origin specific vesicles with different biomarkers or combination of biomarkers, can be performed, such as those disclosed herein.

[00280] Performance of a diagnostic or related test is typically assessed using statistical measures. The performance of the characterization can be assessed by measuring sensitivity, specificity and related measures. For example, a level of circulating biomarkers of interest can be assayed to characterize a phenotype, such as detecting a disease. The sensitivity and specificity of the assay to detect the disease is determined.

[00281] A true positive is a subject with a characteristic, e.g., a disease or disorder, correctly identified as having the characteristic. A false positive is a subject without the characteristic that the test improperly identifies as having the characteristic. A true negative is a subject without the characteristic that the test correctly identifies as not having the characteristic. A false negative is a person with the characteristic that the test improperly identifies as not having the characteristic. The ability of the test to distinguish between these classes provides a measure of test performance.

[00282] The specificity of a test is defined as the number of true negatives divided by the number of actual negatives (i.e., sum of true negatives and false positives). Specificity is a measure of how many subjects are correctly identified as negatives. A specificity of 100% means that the test recognizes all actual negatives - for

PREOMICS EXHIBIT 1002
Page 1002 of 1861

example, all healthy people will be recognized as healthy. A lower specificity indicates that more negatives will be determined as positive.

[00283] The sensitivity of a test is defined as the number of true positives divided by the number of actual positives (i.e., sum of true positives and false negatives). Sensitivity is a measure of how many subjects are correctly identified as positives. A sensitivity of 100% means that the test recognizes all actual positives - for example, all sick people will be recognized as sick. A lower sensitivity indicates that more positives will be missed by being determined as negative.

[00284] The accuracy of a test is defined as the number of true positives and true negatives divided by the sum of all true and false positives and all true and false negatives. It provides one number that combines sensitivity and specificity measurements.

[00285] Sensitivity, specificity and accuracy are determined at a particular discrimination threshold value. For example, a common threshold for prostate cancer (PCa) detection is 4 ng/niL of prostate specific antigen (PSA) in serum. A level of PSA equal to or above the threshold is considered positive for PCa and any level below is considered negative. As the threshold is varied, the sensitivity and specificity will also vary. For example, as the threshold for detecting cancer is increased, the specificity will increase because it is harder to call a subject positive, resulting in fewer false positives. At the same time, the sensitivity will decrease. A receiver operating characteristic curve (ROC curve) is a graphical plot of the true positive rate (i.e., sensitivity) versus the false positive rate (i.e., 1 - specificity) for a binary classifier system as its discrimination threshold is varied. The ROC curve shows how sensitivity and specificity change as the threshold is varied. The Area Under the Curve (AUC) of an ROC curve provides a summary value indicative of a test's performance over the entire range of thresholds. The AUC is equal to the probability that a classifier will rank a randomly chosen positive sample higher than a randomly chosen negative sample. An AUC of 0.5 indicates that the test has a 50% chance of proper ranking, which is equivalent to no discriminatory power (a coin flip also has a 50% chance of proper ranking). An AUC of 1.0 means that the test properly ranks (classifies) all subjects. The AUC is equivalent to the Wilcoxon test of ranks.

[00286] A biosignature according to the invention can be used to characterize a phenotype with at least 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70% sensitivity, such as with at least 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, or 87% sensitivity. In some embodiments, the phenotype is characterized with at least 87.1, 87.2, 87.3, 87.4, 87.5, 87.6, 87.7, 87.8, 87.9, 88.0, or 89% sensitivity, such as at least 90% sensitivity. The phenotype can be characterized with at least 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sensitivity.

[00287] A biosignature according to the invention can be used to characterize a phenotype of a subject with at least 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, or 97% specificity, such as with at least 97.1, 97.2, 97.3, 97.4, 97.5, 97.6, 97.7, 97.8, 97.8, 97.9, 98.0, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99.0, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or 100% specificity.

[00288] A biosignature according to the invention can be used to characterize a phenotype of a subject, e.g., based on a level of a circulating biomarker or other characteristic, with at least 50% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 55% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 60% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100%

PREOMICS EXHIBIT 1002
Page 1003 of 1861

specificity; at least 65% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 70% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 75% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 80% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 85% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 86% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 87% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 88% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 89% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 90% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 91% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 92% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 93% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 94% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 95% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 96% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 97% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 98% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 99% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; or substantially 100% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity.

[00289] A biosignature according to the invention can be used to characterize a phenotype of a subject with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, or 97% accuracy, such as with at least 97.1, 97.2, 97.3, 97.4, 97.5, 97.6, 97.7, 97.8, 97.8, 97.9, 98.0, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99.0, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or 100% accuracy.

[00290] In some embodiments, a biosignature according to the invention is used to characterize a phenotype of a subject with an AUC of at least 0.60, 0.61, 0.62, 0.63, 0.64, 0.65, 0.66, 0.67, 0.68, 0.69, 0.70, 0.71, 0.72, 0.73, 0.74, 0.75, 0.76, 0.77, 0.78, 0.79, 0.80, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, or 0.97, such as with at least 0.971, 0.972, 0.973, 0.974, 0.975, 0.976, 0.977, 0.978, 0.978, 0.979, 0.980, 0.981, 0.982, 0.983, 0.984, 0.985, 0.986, 0.987, 0.988, 0.989, 0.99, 0.991, 0.992, 0.993, 0.994, 0.995, 0.996, 0.997, 0.998, 0.999 or 1.00.

[00291] Furthermore, the confidence level for determining the specificity, sensitivity, accuracy or AUC, may be determined with at least 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% confidence.

[00292] Other related performance measures include positive and negative likelihood ratios [positive LR = sensitivity/(1-specificity); negative LR = (1-sensitivity)/specificity]. Such measures can also be used to gauge test performance according to the methods of the invention.

Classification

[00293] Biosignature according to the invention can be used to classify a sample. Techniques for discriminate analysis are known to those of skill in the art. For example, a sample can be classified as, or predicted to be, a responder or non-responder to a given treatment for a given disease or disorder. Many statistical classification techniques are known to those of skill in the art. In supervised learning approaches, a group of samples from two

PREOMICS EXHIBIT 1002
Page 1004 of 1861

or more groups are analyzed with a statistical classification method. One or more biomarkers, e.g., a panel of biomarkers that forms a biosignature, can be discovered that can be used to build a classifier that differentiates between the two or more groups. A new sample can then be analyzed so that the classifier can associate the new with one of the two or more groups. Commonly used supervised classifiers include without limitation the neural network (multi-layer perceptron), support vector machines, k-nearest neighbors, Gaussian mixture model, Gaussian, naive Bayes, decision tree and radial basis function (RBF) classifiers. Linear classification methods include Fisher's linear discriminant, logistic regression, naive Bayes classifier, perceptron, and support vector machines (SVMs). Other classifiers for use with the invention include quadratic classifiers, k-nearest neighbor, boosting, decision trees, random forests, neural networks, pattern recognition, Bayesian networks and Hidden Markov models. One of skill will appreciate that these or other classifiers, including modifications or improvements of those disclosed herein or known in the art, are contemplated within the scope of the invention.

[00294] Multivariate models that can be used to evaluate a biosignature comprising a presence or level of one or more biomarker include the following:

[00295] Linear discriminant analysis (LDA)

[00296] LDA is a well understood classification method that performs well for cases where predictors follow a generally normal distribution. The method can serve as a benchmark for more complex methods.

[00297] Diagonal linear discriminant analysis (DLDA)

[00298] DLDA is version of discriminant analysis which assumes that predictors are independent, an assumption that may not hold true. However, when training data sets are too small to properly estimate covariances between predictors, well-fit DLDA model may consistently outperform more complex models.

[00299] Shrunken centroids discriminant analysis (SCDA)

[00300] This method is commonly known within the mRNA micorarray community as "PAM" (prediction analysis for microarrays). The method is similar to other for discriminate analysis methods but uses more robust (stabilized) estimates of variance.

[00301] Support vector machines (SVM)

[00302] SVMs are a popular variety of machine learning. SVMs often outperforming traditional statistical methods when predictors are not easily transformed to a multivariate normal distribution. The final SVM model can be expressed in much the same way as an LDA model.

[00303] Tree-based gradient boosting (GBM)

[00304] This method generates binary decision trees, using "boosting" to combine weakly performing trees in a weighted fashion to form a stronger ensemble.

[00305] Lasso (Lasso)

[00306] This approach fits a logistic regression model using "lasso" penalized maximum likelihood method. This approach tends to pick one representative marker from a set of highly correlated markers, returning zero values for coefficients of the remaining markers.

[00307] A classifier's performance can be estimated using a "training" set of sample to build a classifier and an independent "test" set of samples to test the model. Other techniques can be used in the art to estimate predictive performance, such as cross-validation methods. One round of cross-validation involves partitioning a sample of data into complementary subsets, performing the analysis on one subset (the training set), and validating the analysis on the other subset (the validation set or testing set). To reduce variability, multiple rounds of cross-

PREOMICS EXHIBIT 1002
Page 1005 of 1861

validation can be performed using different partitions, and the validation results are averaged over the rounds. Common types of cross-validation include the following:

[00308] K-fold cross-validation

[00309] The sample group is partitioned into k-partitions. One partition is used as the test set and the remainder are used as the training set. The process is repeated k times (or k folds) using each of the partitions once as the test set. The performance of the classifier model is averaged over the iterations. 10-fold cross validation is common though other numbers can be selected depending on sample size, computational resources, and the like.

[00310] 2-fold cross-validation

[00311] This is the simplest version of k-fold validation wherein the data is split into two equal size groups and each group is used for alternate rounds of training and testing.

[00312] Leave-one-out cross-validation

[00313] In this approach, a single sample is withdrawn from the cohort for testing and the rest of the samples are used for training. If each sample is used once as the test sample, this approach is a form of k-folds cross validation where the number of iterations equals the number of samples.

[00314] Repeated random sub-sampling validation

[00315] In this approach, random subsets are drawn for the training and test set for each round of testing. As a result, each sample may not be used for both testing and training over the course of validation.

[00316] Classification using supervised methods is generally performed by the following methodology:

[00317] In order to solve a given problem of supervised learning (e.g. learning to distinguish between two biological states) one generally considers various steps:

[00318] 1. Gather a training set. These can include, for example, samples that are from a subject with or without a disease or disorder, subjects that are known to respond or not respond to a treatment, subjects whose disease progresses or does not progress, etc. The training samples are used to "train" the classifier.

[00319] 2. Determine the input "feature" representation of the learned function. The accuracy of the learned function depends on how the input object is represented. Typically, the input object is transformed into a feature vector, which contains a number of features that are descriptive of the object. The number of features should not be too large, because of the curse of dimensionality; but should be large enough to accurately predict the output. The features might include a set of biomarkers such as those described herein.

[00320] 3. Determine the structure of the learned function and corresponding learning algorithm. A learning algorithm is chosen, e.g., artificial neural networks, decision trees, Bayes classifiers or support vector machines. The learning algorithm is used to build the classifier.

[00321] 4. Build the classifier. The learning algorithm is run the gathered training set. Parameters of the learning algorithm may be adjusted by optimizing performance on a subset (called a validation set) of the training set, or via cross-validation. After parameter adjustment and learning, the performance of the algorithm may be measured on a test set of naive samples that is separate from the training set.

[00322] Once the classifier is determined as described above, it can be used to classify a sample, e.g., that of a subject who is being analyzed by the methods of the invention. As an example, a classifier can be built using data for levels of circulating biomarkers of interest in reference subjects with and without a disease as the training and test sets. Circulating biomarker levels found in a sample from a test subject are assessed and the classifier is used to classify the subject as with or without the disease. As another example, a classifier can be

PREOMICS EXHIBIT 1002
Page 1006 of 1861

built using data for levels of vesicle biomarkers of interest in reference subjects that have been found to respond or not respond to certain diseases as the training and test sets. The vesicle biomarker levels found in a sample from a test subject are assessed and the classifier is used to classify the subject as with or without the disease.

[00323] Unsupervised learning approaches can also be used with the invention. Clustering is an unsupervised learning approach wherein a clustering algorithm correlates a series of samples without the use the labels. The most similar samples are sorted into "clusters." A new sample could be sorted into a cluster and thereby classified with other members that it most closely associates. Many clustering algorithms well known to those of skill in the art can be used with the invention, such as hierarchical clustering.

Biosignatures

[00324] A biosignature can be obtained according to the invention by assessing a vesicle population, including surface and payload vesicle associated biomarkers, and/or circulating biomarkers including microRNA and protein. A biosignature derived from a subject can be used to characterize a phenotype of the subject. A biosignature can further include the level of one or more additional biomarkers, e.g., circulating biomarkers or biomarkers associated with a vesicle of interest. A biosignature of a vesicle of interest can include particular antigens or biomarkers that are present on the vesicle. The biosignature can also include one or more antigens or biomarkers that are carried as payload within the vesicle, including the microRNA under examination. The biosignature can comprise a combination of one or more antigens or biomarkers that are present on the vesicle with one or more biomarkers that are detected in the vesicle. The biosignature can further comprise other information about a vesicle aside from its biomarkers. Such information can include vesicle size, circulating half-life, metabolic half-life, and specific activity in vivo or in vitro. The biosignature can comprise the biomarkers or other characteristics used to build a classifier.

[00325] In some embodiments, the microRNA is detected directly in a biological sample. For example, RNA in a bodily fluid can be isolated using commercially available kits such as *mirVana* kits (Applied Biosystems/ Ambion, Austin, TX), MagMAX™ RNA Isolation Kit (Applied Biosystems/ Ambion, Austin, TX), and QIAzol Lysis Reagent and RNeasy Midi Kit (Qiagen Inc., Valencia CA). Particular species of microRNAs can be determined using array or PCR techniques as described below.

[00326] In some embodiments, the microRNA payload with vesicles is assessed in order to characterize a phenotype. The vesicles can be purified or concentrated prior to determining the biosignature. For example, a cell-of-origin specific vesicle can be isolated and its biosignature determined. Alternatively, the biosignature of the vesicle can be directly assayed from a sample, without prior purification or concentration. The biosignature of the invention can be used to determine a diagnosis, prognosis, or theranosis of a disease or condition or similar measures described herein. A biosignature can also be used to determine treatment efficacy, stage of a disease or condition, or progression of a disease or condition, or responder / non-responder status. Furthermore, a biosignature may be used to determine a physiological state, such as pregnancy.

[00327] A characteristic of a vesicle in and of itself can be assessed to determine a biosignature. The characteristic can be used to diagnose, detect or determine a disease stage or progression, the therapeutic implications of a disease or condition, or characterize a physiological state. Such characteristics include without limitation the level or amount of vesicles, vesicle size, temporal evaluation of the variation in vesicle half-life, circulating vesicle half-life, metabolic half-life of a vesicle, or activity of a vesicle.

PREOMICS EXHIBIT 1002
Page 1007 of 1861

[00328] Biomarkers that can be included in a biosignature include one or more proteins or peptides (e.g., providing a protein signature), nucleic acids (e.g. RNA signature as described, or a DNA signature), lipids (e.g. lipid signature), or combinations thereof. In some embodiments, the biosignature can also comprise the type or amount of drug or drug metabolite present in a vesicle, (e.g., providing a drug signature), as such drug may be taken by a subject from which the biological sample is obtained, resulting in a vesicle carrying the drug or metabolites of the drug.

[00329] A biosignature can also include an expression level, presence, absence, mutation, variant, copy number variation, truncation, duplication, modification, or molecular association of one or more biomarkers. A genetic variant, or nucleotide variant, refers to changes or alterations to a gene or cDNA sequence at a particular locus, including, but not limited to, nucleotide base deletions, insertions, inversions, and substitutions in the coding and non-coding regions. Deletions may be of a single nucleotide base, a portion or a region of the nucleotide sequence of the gene, or of the entire gene sequence. Insertions may be of one or more nucleotide bases. The genetic variant may occur in transcriptional regulatory regions, untranslated regions of mRNA, exons, introns, or exon/intron junctions. The genetic variant may or may not result in stop codons, frame shifts, deletions of amino acids, altered gene transcript splice forms or altered amino acid sequence.

[00330] In an embodiment, nucleic acid biomarkers, including nucleic acid payload within a vesicle, is assessed for nucleotide variants. The nucleic acid biomarker may comprise one or more RNA species, e.g., mRNA, miRNA, snoRNA, snRNA, rRNAs, tRNAs, siRNA, hnRNA, shRNA, enhancer RNA (eRNA), or a combination thereof. Similarly, DNA payload can be assessed to form a DNA signature.

[00331] An RNA signature or DNA signature can also include a mutational, epigenetic modification, or genetic variant analysis of the RNA or DNA present in the vesicle. Epigenetic modifications include patterns of DNA methylation. See, e.g., Lesche R. and Eckhardt F., DNA methylation markers: a versatile diagnostic tool for routine clinical use. Curr Opin Mol Ther. 2007 Jun;9(3):222-30, which is incorporated herein by reference in its entirety. Thus, a biomarker can be the methylation status of a segment of DNA.

[00332] A biosignature can comprise one or more miRNA signatures combined with one or more additional signatures including, but not limited to, an mRNA signature, DNA signature, protein signature, peptide signature, antigen signature, or any combination thereof. For example, the biosignature can comprise one or more miRNA biomarkers with one or more DNA biomarkers, one or more mRNA biomarkers, one or more snoRNA biomarkers, one or more protein biomarkers, one or more peptide biomarkers, one or more antigen biomarkers, one or more antigen biomarkers, one or more lipid biomarkers, or any combination thereof.

[00333] A biosignature can comprise a combination of one or more antigens or binding agents (such as ability to bind one or more binding agents), such as listed in **Figs. 1** and **2,** respectively, of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein, or those described elsewhere herein. The biosignature can further comprise one or more other biomarkers, such as, but not limited to, miRNA, DNA (e.g. single stranded DNA, complementary DNA, or noncoding DNA), or mRNA. The biosignature of a vesicle can comprise a combination of one or more antigens, such as shown in **Fig. 1** of International Patent Application Serial No. PCT/US201 1/03 1479, one or more binding agents, such as shown in **Fig. 2** of International Patent Application Serial No. PCT/US201 1/03 1479, and one or more biomarkers for a condition or disease, such as listed in **Figs. 3-60** of International Patent Application Serial No. PCT/US201 1/03 1479. The biosignature can

PREOMICS EXHIBIT 1002
Page 1008 of 1861

comprise one or more biomarkers, for example miRNA, with one or more antigens specific for a cancer cell (for example, as shown in **Fig. 1** of International Patent Application Serial No. PCT/US201 1/03 1479).

[00334] In some embodiments, a vesicle used in the subject methods has a biosignature that is specific to the cell-of-origin and is used to derive disease-specific or biological state specific diagnostic, prognostic or therapy-related biosignatures representative of the cell-of-origin. In other embodiments, a vesicle has a biosignature that is specific to a given disease or physiological condition that is different from the biosignature of the cell-of-origin for use in the diagnosis, prognosis, staging, therapy-related determinations or physiological state characterization. Biosignatures can also comprise a combination of cell-of-origin specific and non-specific vesicles.

[00335] Biosignatures can be used to evaluate diagnostic criteria such as presence of disease, disease staging, disease monitoring, disease stratification, or surveillance for detection, metastasis or recurrence or progression of disease. A biosignature can also be used clinically in making decisions concerning treatment modalities including therapeutic intervention. A biosignature can further be used clinically to make treatment decisions, including whether to perform surgery or what treatment standards should be used along with surgery (e.g., either pre-surgery or post-surgery). As an illustrative example, a biosignature of circulating biomarkers that indicates an aggressive form of cancer may call for a more aggressive surgical procedure and/or more aggressive therapeutic regimen to treat the patient.

[00336] A biosignature can be used in therapy related diagnostics to provide tests useful to diagnose a disease or choose the correct treatment regimen, such as provide a theranosis. Theranostics includes diagnostic testing that provides the ability to affect therapy or treatment of a diseased state. Theranostics testing provides a theranosis in a similar manner that diagnostics or prognostic testing provides a diagnosis or prognosis, respectively. As used herein, theranostics encompasses any desired form of therapy related testing, including predictive medicine, personalized medicine, integrated medicine, pharmacodiagnostics and Dx/Rx partnering. Therapy related tests can be used to predict and assess drug response in individual subjects, i.e., to provide personalized medicine. Predicting a drug response can be determining whether a subject is a likely responder or a likely non-responder to a candidate therapeutic agent, e.g., before the subject has been exposed or otherwise treated with the treatment. Assessing a drug response can be monitoring a response to a drug, e.g., monitoring the subject's improvement or lack thereof over a time course after initiating the treatment. Therapy related tests are useful to select a subject for treatment who is particularly likely to benefit from the treatment or to provide an early and objective indication of treatment efficacy in an individual subject. Thus, a biosignature as disclosed herein may indicate that treatment should be altered to select a more promising treatment, thereby avoiding the great expense of delaying beneficial treatment and avoiding the financial and morbidity costs of administering an ineffective drug(s).

[00337] Therapy related diagnostics are also useful in clinical diagnosis and management of a variety of diseases and disorders, which include, but are not limited to cardiovascular disease, cancer, infectious diseases, sepsis, neurological diseases, central nervous system related diseases, endovascular related diseases, and autoimmune related diseases. Therapy related diagnostics also aid in the prediction of drug toxicity, drug resistance or drug response. Therapy related tests may be developed in any suitable diagnostic testing format, which include, but are not limited to, e.g., immunohistochemical tests, clinical chemistry, immunoassay, cell-based technologies, nucleic acid tests or body imaging methods. Therapy related tests can further include but are

PREOMICS EXHIBIT 1002
Page 1009 of 1861

not limited to, testing that aids in the determination of therapy, testing that monitors for therapeutic toxicity, or response to therapy testing. Thus, a biosignature can be used to predict or monitor a subject's response to a treatment. A biosignature can be determined at different time points for a subject after initiating, removing, or altering a particular treatment.

[00338] In some embodiments, a determination or prediction as to whether a subject is responding to a treatment is made based on a change in the amount of one or more components of a biosignature (i.e., the microRNA, vesicles and/or biomarkers of interest), an amount of one or more components of a particular biosignature, or the biosignature detected for the components. In another embodiment, a subject's condition is monitored by determining a biosignature at different time points. The progression, regression, or recurrence of a condition is determined. Response to therapy can also be measured over a time course. Thus, the invention provides a method of monitoring a status of a disease or other medical condition in a subject, comprising isolating or detecting a biosignature from a biological sample from the subject, detecting the overall amount of the components of a particular biosignature, or detecting the biosignature of one or more components (such as the presence, absence, or expression level of a biomarker). The biosignatures are used to monitor the status of the disease or condition.

[00339] One or more novel biosignatures of a vesicle can also be identified. For example, one or more vesicles can be isolated from a subject that responds to a drug treatment or treatment regimen and compared to a reference, such as another subject that does not respond to the drug treatment or treatment regimen. Differences between the biosignatures can be determined and used to identify other subjects as responders or non-responders to a particular drug or treatment regimen.

[00340] In some embodiments, a biosignature is used to determine whether a particular disease or condition is resistant to a drug. If a subject is drug resistant, a physician need not waste valuable time with such drug treatment. To obtain early validation of a drug choice or treatment regimen, a biosignature is determined for a sample obtained from a subject. The biosignature is used to assess whether the particular subject's disease has the biomarker associated with drug resistance. Such a determination enables doctors to devote critical time as well as the patient's financial resources to effective treatments.

[00341] Moreover, biosignature may be used to assess whether a subject is afflicted with disease, is at risk for developing disease or to assess the stage or progression of the disease. For example, a biosignature can be used to assess whether a subject has prostate cancer, colon cancer, or other cancer as described herein. Futhermore, a biosignature can be used to determine a stage of a disease or condition, such as colon cancer.

[00342] Furthermore, determining the amount of vesicles, such a heterogeneous population of vesicles, and the amount of one or more homogeneous population of vesicles, such as a population of vesicles with the same biosignature, can be used to characterize a phenotype. For example, determination of the total amount of vesicles in a sample (i.e. not cell-type specific) and determining the presence of one or more different cell-of-origin specific vesicles can be used to characterize a phenotype. Threshold values, or reference values or amounts can be determined based on comparisons of normal subjects and subjects with the phenotype of interest, as further described below, and criteria based on the threshold or reference values determined. The different criteria can be used to characterize a phenotype.

[00343] One criterion can be based on the amount of a heterogeneous population of vesicles in a sample. In one embodiment, general vesicle markers, such as CD9, CD81, and CD63 can be used to determine the amount of

PREOMICS EXHIBIT 1002
Page 1010 of 1861

vesicles in a sample. The expression level of CD9, CD81, CD63, or a combination thereof can be detected and if the level is greater than a threshold level, the criterion is met. In another embodiment, the criterion is met if if level of CD9, CD81, CD63, or a combination thereof is lower than a threshold value or reference value. In another embodiment, the criterion can be based on whether the amount of vesicles is higher than a threshold or reference value. Another criterion can be based on the amount of vesicles with a specific biosignature. If the amount of vesicles with the specific biosignature is lower than a threshold or reference value, the criterion is met. In another embodiment, if the amount of vesicles with the specific biosignature is higher than a threshold or reference value, the criterion is met. A criterion can also be based on the amount of vesicles derived from a particular cell type. If the amount is lower than a threshold or reference value, the criterion is met. In another embodiment, if the amount is higher than a threshold value, the criterion is met.

[00344] In a non-limiting example, consider that vesicles from prostate cells are determined by detecting the biomarker PCSA or PSCA, and that a criterion is met if the level of detected PCSA or PSCA is greater than a threshold level. The threshold can be the level of the same markers in a sample from a control cell line or control subject. Another criterion can be based on whether the amount of vesicles derived from a cancer cell or comprising one or more cancer specific biomarkers. For example, the biomarkers B7H3, EpCam, or both, can be determined and a criterion met if the level of detected B7H3 and/or EpCam is greater than a threshold level or within a pre-determined range. If the amount is lower, or higher, than a threshold or reference value, the criterion is met. A criterion can also be the reliability of the result, such as meeting a quality control measure or value. A detected amount of B7H3 and/or EpCam in a test sample that is above the amount of these markers in a control sample may indicate the presence of a cancer in the test sample.

[00345] As described, analysis of multiple markers can be combined to assess whether a criterion is met. In an illustrative example, a biosignature is used to assess whether a subject has prostate cancer by detecting one or more of the general vesicle markers CD9, CD63 and CD81 ; one or more prostate epithelial markers including PCSA or PSMA; and one or more cancer markers such as B7H3 and/or EpCam. Higher levels of the markers in a sample from a subject than in a control individual without prostate cancer indicates the presence of the prostate cancer in the subject. In some embodiments, the multiple markers are assessed in a multiplex fashion.

[00346] One of skill will understand that such rules based on meeting criterion as described can be applied to any appropriate biomarker. For example, the criterion can be applied to vesicle characteristics such as amount of vesicles present, amount of vesicles with a particular biosignature present, amount of vesicle payload biomarkers present, amount of microRNA or other circulating biomarkers present, and the like. The ratios of appropriate biomarkers can be determined. As illustrative examples, the criterion could be a ratio of an vesicle surface protein to another vesicle surface protein, a ratio of an vesicle surface protein to a microRNA, a ratio of one vesicle population to another vesicle population, a ratio of one circulating biomarker to another circulating biomarker, etc.

[00347] A phenotype for a subject can be characterized based on meeting any number of useful criteria. In some embodiments, at least one criterion is used for each biomarker. In some embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 or at least 100 criteria are used. For example, for the characterizing of a cancer, a number of different criteria can be used when the subject is diagnosed with a cancer: 1) if the amount of microRNA in a sample from a subject is higher than a reference value; 2) if the amount of a microRNA within cell type specific vesicles (i.e. vesicles derived from a specific tissue or organ) is

PREOMICS EXHIBIT 1002
Page 1011 of 1861

higher than a reference value; or 3) if the amount of microRNA within vesicles with one or more cancer specific biomarkers is higher than a reference value. Similar rules can apply if the amount of microRNA is less than or the same as the reference. The method can further include a quality control measure, such that the results are provided for the subject if the samples meet the quality control measure. In some embodiments, if the criteria are met but the quality control is questionable, the subject is reassessed.

[00348] In other embodiments, a single measure is determined for assessment of multiple biomarkers, and the measure is compared to a reference. For illustration, a test for prostate cancer might comprise multiplying the level of PSA against the level of miR-141 in a blood sample. The criterion is met if the product of the levels is above a threshold, indicating the presense of the cancer. As another illustration, a number of binding agents to general vesicle markers can carry the same label, e.g., the same fluorophore. The level of the detected label can be compared to a threshold.

[00349] Criterion can be applied to multiple types of biomarkers in addition to multiple biomarkers of the same type. For example, the levels of one or more circulating biomarkers (e.g., RNA, DNA, peptides), vesicles, mutations, etc, can be compared to a reference. Different components of a biosignature can have different criteria. As a non-limiting example, a biosignature used to diagnose a cancer can include overexpression of one miR species as compared to a reference and underexpression of a vesicle surface antigen as compared to another reference.

[00350] A biosignature can be determined by comparing the amount of vesicles, the structure of a vesicle, or any other informative characteristic of a vesicle. Vesicle structure can be assessed using transmission electron microscopy, see for example, *Hansen et al., Journal of Biomechanics 31, Supplement 1: 134-134(1) (1998),* or scanning electron microscopy. Various combinations of methods and techniques or analyzing one or more vesicles can be used to determine a phenotype for a subject.

[00351] A biosignature can include without limitation the presence or absence, copy number, expression level, or activity level of a biomarker. Other useful components of a biosignature include the presence of a mutation (e.g., mutations which affect activity of a transcription or translation product, such as substitution, deletion, or insertion mutations), variant, or post-translation modification of a biomarker. Post-translational modification of a protein biomarker include without limitation acylation, acetylation, phosphorylation, ubiquitination, deacetylation, alkylation, methylation, amidation, biotinylation, gamma-carboxylation, glutamylation, glycosylation, glycyation, hydroxylation, covalent attachment of heme moiety, iodination, isoprenylation, lipoylation, prenylation, GPI anchor formation, myristoylation, farnesylation, geranylgeranylation, covalent attachment of nucleotides or derivatives thereof, ADP-ribosylation, flavin attachment, oxidation, palmitoylation, pegylation, covalent attachment of phosphatidylinositol, phosphopantetheinylation, polysialylation, pyroglutamate formation, racemization of proline by prolyl isomerase, tRNA-mediation addition of amino acids such as arginylation, sulfation, the addition of a sulfate group to a tyrosine, or selenoylation of the biomarker.

[00352] The methods described herein can be used to identify a biosignature that is associated with a disease, condition or physiological state. The biosignature can also be used to determine if a subject is afflicted with cancer or is at risk for developing cancer. A subject at risk of developing cancer can include those who may be predisposed or who have pre-symptomatic early stage disease.

[00353] A biosignature can also be used to provide a diagnostic or theranostic determination for other diseases including but not limited to autoimmune diseases, inflammatory bowel diseases, cardiovascular disease,

PREOMICS EXHIBIT 1002
Page 1012 of 1861

neurological disorders such as Alzheimer's disease, Parkinson's disease, Multiple Sclerosis, sepsis or pancreatitis or any disease, conditions or symptoms listed in **Figs. 3-58** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein.

[00354] The biosignature can also be used to identify a given pregnancy state from the peripheral blood, umbilical cord blood, or amniotic fluid (e.g. miRNA signature specific to Downs Syndrome) or adverse pregnancy outcome such as pre-eclampsia, pre-term birth, premature rupture of membranes, intrauterine growth restriction or recurrent pregnancy loss. The biosignature can also be used to indicate the health of the mother, the fetus at all developmental stages, the pre-implantation embryo or a newborn.

[00355] A biosignature can be used for pre-symptomatic diagnosis. Furthermore, the biosignature can be used to detect disease, determine disease stage or progression, determine the recurrence of disease, identify treatment protocols, determine efficacy of treatment protocols or evaluate the physiological status of individuals related to age and environmental exposure.

[00356] Monitoring a biosignature of a vesicle can also be used to identify toxic exposures in a subject including, but not limited to, situations of early exposure or exposure to an unknown or unidentified toxic agent. Without being bound by any one specific theory for mechanism of action, vesicles can shed from damaged cells and in the process compartmentalize specific contents of the cell including both membrane components and engulfed cytoplasmic contents. Cells exposed to toxic agents/chemicals may increase vesicle shedding to expel toxic agents or metabolites thereof, thus resulting in increased vesicle levels. Thus, monitoring vesicle levels, vesicle biosignature, or both, allows assessment of an individual's response to potential toxic agent(s).

[00357] A vesicle and/or other biomarkers of the invention can be used to identify states of drug-induced toxicity or the organ injured, by detecting one or more specific antigen, binding agent, biomarker, or any combination thereof. The level of vesicles, changes in the biosignature of a vesicle, or both, can be used to monitor an individual for acute, chronic, or occupational exposures to any number of toxic agents including, but not limited to, drugs, antibiotics, industrial chemicals, toxic antibiotic metabolites, herbs, household chemicals, and chemicals produced by other organisms, either naturally occurring or synthetic in nature. In addition, a biosignature can be used to identify conditions or diseases, including cancers of unknown origin, also known as cancers of unknown primary (CUP).

[00358] A vesicle may be isolated from a biological sample as previously described to arrive at a heterogeneous population of vesicles. The heterogeneous population of vesicles can then be contacted with substrates coated with specific binding agents designed to rule out or identify antigen specific characteristics of the vesicle population that are specific to a given cell-of-origin. Further, as described above, the biosignature of a vesicle can correlate with the cancerous state of cells. Compounds that inhibit cancer in a subject may cause a change, e.g., a change in biosignature of a vesicle, which can be monitored by serial isolation of vesicles over time and treatment course. The level of vesicles or changes in the level of vesicles with a specific biosignature can be monitored.

[00359] In an aspect, characterizing a phenotype of a subject comprises a method of determining whether the subject is likely to respond or not respond to a therapy. The methods of the invention also include determining new biosignatures useful in predicting whether the subject is likely to respond or not. One or more subjects that respond to a therapy (responders) and one or more subjects that do not respond to the same therapy (non-

PREOMICS EXHIBIT 1002
Page 1013 of 1861

responders) can have their vesicles interrogated. Interrogation can be performed to identify vesicle biosignatures that classify a subject as a responder or non-responder to the treatment of interest. In some aspects, the presence, quantity, and payload of a vesicle are assayed. The payload of a vesicle includes, for example, internal proteins, nucleic acids such as miRNA, lipids or carbohydrates.

[00360] The presence or absence of a biosignature in responders but not in the non-responders can be used for theranosis. A sample from responders may be analyzed for one or more of the following: amount of vesicles, amount of a unique subset or species of vesicles, biomarkers in such vesicles, biosignature of such vesicles, etc. In one instance, vesicles such as microvesicles or exosomes from responders and non-responders are analyzed for the presence and/or quantity of one or more miRNAs, such as miRNA 122, miR-548c-5p, miR-362-3p, miR-422a, miR-597, miR-429, miR-200a, and/or miR-200b. A difference in biosignatures between responders and non-responders can be used for theranosis. In another embodiment, vesicles are obtained from subjects having a disease or condition. Vesicles are also obtained from subjects free of such disease or condition. The vesicles from both groups of subjects are assayed for unique biosignatures that are associated with all subjects in that group but not in subjects from the other group. Such biosignatures or biomarkers can then used as a diagnostic for the presence or absence of the condition or disease, or to classify the subject as belonging on one of the groups (those with/without disease, aggressive/non-aggressive disease, responder/non-responder, etc).

[00361] In an aspect, characterizing a phenotype of a subject comprises a method of staging a disease. The methods of the invention also include determining new biosignatures useful in staging. In an illustrative example, vesicles are assayed from patients having a stage I cancer and patients having stage II or stage III of the same cancer. In some embodiments, vesicles are assayed in patients with metastatic disease. A difference in biosignatures or biomarkers between vesicles from each group of patient is identified (e.g., vesicles from stage III cancer may have an increased expression of one or more genes or miRNA' s), thereby identifying a biosignature or biomarker that distinguishes different stages of a disease. Such biosignature can then be used to stage patients having the disease.

[00362] In some instances, a biosignature is determined by assaying vesicles from a subject over a period of time, e.g., daily, semiweekly, weekly, biweekly, semimonthly, monthly, bimonthly, semiquarterly, quarterly, semiyearly, biyearly or yearly. For example, the biosignatures in patients on a given therapy can be monitored over time to detect signatures indicative of responders or non-responders for the therapy. Similarly, patients with differing stages of disease or in differing stages of a clinical trial have a biosignature interrogated over time. The payload or physical attributes of the vesicles in each point in time can be compared. A temporal pattern can thus form a biosignature that can then be used for theranosis, diagnosis, prognosis, disease stratification, treatment monitoring, disease monitoring or making a prediction of responder / non-responder status. As an illustrative example only, an increasing amount of a biomarker (e.g., miR 122) in vesicles over a time course is associated with metastatic cancer, as opposed to a stagnant amounts of the biomarker in vesicles over the time course that are associated with non-metastatic cancer. A time course may last over at least 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 6 weeks, 8 weeks, 2 months, 10 weeks, 12 weeks, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, one year, 18 months, 2 years, or at least 3 years.

[00363] The level of vesicles, level of vesicles with a specific biosignature, or a biosignature of a vesicle can also be used to assess the efficacy of a therapy for a condition. For example, the level of vesicles, level of

PREOMICS EXHIBIT 1002
Page 1014 of 1861

vesicles with a specific biosignature, or a biosignature of a vesicle can be used to assess the efficacy of a cancer treatment, e.g., chemotherapy, radiation therapy, surgery, or any other therapeutic approach useful for inhibiting cancer in a subject. In addition, a biosignature can be used in a screening assay to identify candidate or test compounds or agents (e.g., proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) that have a modulatory effect on the biosignature of a vesicle. Compounds identified via such screening assays may be useful, for example, for modulating, e.g., inhibiting, ameliorating, treating, or preventing conditions or diseases.

[00364] For example, a biosignature for a vesicle can be obtained from a patient who is undergoing successful treatment for a particular cancer. Cells from a cancer patient not being treated with the same drug can be cultured and vesicles from the cultures obtained for determining biosignatures. The cells can be treated with test compounds and the biosignature of the vesicles from the cultures can be compared to the biosignature of the vesicles obtained from the patient undergoing successful treatment. The test compounds that results in biosignatures that are similar to those of the patient undergoing successful treatment can be selected for further studies.

[00365] The biosignature of a vesicle can also be used to monitor the influence of an agent (e.g., drug compounds) on the biosignature in clinical trials. Monitoring the level of vesicles, changes in the biosignature of a vesicle, or both, can also be used in a method of assessing the efficacy of a test compound, such as a test compound for inhibiting cancer cells.

[00366] In addition to diagnosing or confirming the presence of or risk for developing a disease, condition or a syndrome, the methods and compositions disclosed herein also provide a system for optimizing the treatment of a subject having such a disease, condition or syndrome. The level of vesicles, the biosignature of a vesicle, or both, can also be used to determine the effectiveness of a particular therapeutic intervention (pharmaceutical or non-pharmaceutical) and to alter the intervention to 1) reduce the risk of developing adverse outcomes, 2) enhance the effectiveness of the intervention or 3) identify resistant states. Thus, in addition to diagnosing or confirming the presence of or risk for developing a disease, condition or a syndrome, the methods and compositions disclosed herein also provide a system for optimizing the treatment of a subject having such a disease, condition or syndrome. For example, a therapy-related approach to treating a disease, condition or syndrome by integrating diagnostics and therapeutics to improve the real-time treatment of a subject can be determined by identifying the biosignature of a vesicle.

[00367] Tests that identify the level of vesicles, the biosignature of a vesicle, or both, can be used to identify which patients are most suited to a particular therapy, and provide feedback on how well a drug is working, so as to optimize treatment regimens. For example, in pregnancy-induced hypertension and associated conditions, therapy-related diagnostics can flexibly monitor changes in important parameters (e.g., cytokine and/or growth factor levels) over time, to optimize treatment.

[00368] Within the clinical trial setting of investigational agents as defined by the FDA, MDA, EMA, USDA, and EMEA, therapy-related diagnostics as determined by a biosignature disclosed herein, can provide key information to optimize trial design, monitor efficacy, and enhance drug safety. For instance, for trial design, therapy-related diagnostics can be used for patient stratification, determination of patient eligibility (inclusion/exclusion), creation of homogeneous treatment groups, and selection of patient samples that are optimized to a matched case control cohort. Such therapy-related diagnostic can therefore provide the means for

PREOMICS EXHIBIT 1002
Page 1015 of 1861

patient efficacy enrichment, thereby minimizing the number of individuals needed for trial recruitment. For example, for efficacy, therapy-related diagnostics are useful for monitoring therapy and assessing efficacy criteria. Alternatively, for safety, therapy-related diagnostics can be used to prevent adverse drug reactions or avoid medication error and monitor compliance with the therapeutic regimen.

[00369] In some embodiments, the invention provides a method of identifying responder and non-responders to a treatment undergoing clinical trials, comprising detecting biosignatures comprising circulating biomarkers in subjects enrolled in the clinical trial, and identifying biosignatures that distinguish between responders and non-responders. In a further embodiment, the biosignatures are measured in a drug naive subject and used to predict whether the subject will be a responder or non-responder. The prediction can be based upon whether the biosignatures of the drug naive subject correlate more closely with the clinical trial subjects identified as responders, thereby predicting that the drug naive subject will be a responder. Conversely, if the biosignatures of the drug naive subject correlate more closely with the clinical trial subjects identified as non-responders, the methods of the invention can predict that the drug naive subject will be a non-responder. The prediction can therefore be used to stratify potential responders and non-responders to the treatment. In some embodiments, the prediction is used to guide a course of treatment, e.g., by helping treating physicians decide whether to administer the drug. In some embodiments, the prediction is used to guide selection of patients for enrollment in further clinical trials. In a non-limiting example, biosignatures that predict responder / non-responder status in Phase II trials can be used to select patients for a Phase III trial, thereby increasing the likelihood of response in the Phase III patient population. One of skill will appreciate that the method can be adapted to identify biosignatures to stratify subjects on criteria other than responder / non-responder status. In one embodiment, the criterion is treatment safety. Therefore the method is followed as above to identify subjects who are likely or not to have adverse events to the treatment. In a non-limiting example, biosignatures that predict safety profile in Phase II trials can be used to select patients for a Phase III trial, thereby increasing the treatment safety profile in the Phase III patient population.

[00370] Therefore, the level of vesicles, the biosignature of a vesicle, or both, can be used to monitor drug efficacy, determine response or resistance to a given drug, or both, thereby enhancing drug safety. For example, in colon cancer, vesicles are typically shed from colon cancer cells and can be isolated from the peripheral blood and used to isolate one or more biomarkers e.g., KRAS mRNA which can then be sequenced to detect KRAS mutations. In the case of mRNA biomarkers, the mRNA can be reverse transcribed into cDNA and sequenced (e.g., by Sanger sequencing, pyrosequencing, NextGen sequencing, RT-PCR assays) to determine if there are mutations present that confer resistance to a drug (e.g., cetuximab or panitumimab). In another example, vesicles that are specifically shed from lung cancer cells are isolated from a biological sample and used to isolate a lung cancer biomarker, e.g., EGFR mRNA. The EGFR mRNA is processed to cDNA and sequenced to determine if there are EGFR mutations present that show resistance or response to specific drugs or treatments for lung cancer.

[00371] One or more biosignatures can be grouped so that information obtained about the set of biosignatures in a particular group provides a reasonable basis for making a clinically relevant decision, such as but not limited to a diagnosis, prognosis, or management of treatment, such as treatment selection.

PREOMICS EXHIBIT 1002
Page 1016 of 1861

[00372] As with most diagnostic markers, it is often desirable to use the fewest number of markers sufficient to make a correct medical judgment. This prevents a delay in treatment pending further analysis as well inappropriate use of time and resources.

[00373] Also disclosed herein are methods of conducting retrospective analysis on samples (e.g., serum and tissue biobanks) for the purpose of correlating qualitative and quantitative properties, such as biosignatures of vesicles, with clinical outcomes in terms of disease state, disease stage, progression, prognosis; therapeutic efficacy or selection; or physiological conditions. Furthermore, methods and compositions disclosed herein are used for conducting prospective analysis on a sample (e.g., serum and/or tissue collected from individuals in a clinical trial) for the purpose of correlating qualitative and quantitative biosignatures of vesicleswith clinical outcomes in terms of disease state, disease stage, progression, prognosis; therapeutic efficacy or selection; or physiological conditions can also be performed. As used herein, a biosignature for a vesicle can be used to identify a cell-of-origin specific vesicle. Furthermore, a biosignature can be determined based on a surface marker profile of a vesicle or contents of a vesicle.

[00374] The biosignatures used to characterize a phenotype according to the invention can comprise multiple components (e.g., microRNA, vesicles or other biomarkers) or characteristics (e.g., vesicle size or morphology). The biosignatures can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, or 100 components or characteristics. A biosignature with more than one component or characteristic, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, or 100 components, may provide higher sensitivity and/or specificity in characterizing a phenotype. In some embodiments, assessing a plurality of components or characteristics provides increased sensitivity and/or specificity as compared to assessing fewer components or characteristics. On the other hand, it is often desirable to use the fewest number of components or characteristics sufficient to make a correct medical judgment. Fewer markers can avoid statistical overfitting of a classifier and can prevent a delay in treatment pending further analysis as well inappropriate use of time and resources. Thus, the methods of the invention comprise determining an optimal number of components or characteristics.

[00375] A biosignature according to the invention can be used to characterize a phenotype with a sensitivity, specificity, accuracy, or similar performance metric as described above. The biosignatures can also be used to build a classifier to classify a sample as belonging to a group, such as belonging to a group having a disease or not, a group having an aggressive disease or not, or a group of responders or non-responders. In one embodiment, a classifier is used to determine whether a subject has an aggressive or non-aggressive cancer. In the illustrative case of prostate cancer, this can help a physician to determine whether to watch the cancer, i.e., prescribe "watchful waiting," or perform a prostatectomy. In another embodiment, a classifier is used to determine whether a breast cancer patient is likely to respond or not to tamoxifen, thereby helping the physician to determine whether or not to treat the patient with tamoxifen or another drug.

Biomarkers

[00376] A biosignature used to characterize a phenotype can comprise one or more biomarkers. The biomarker can be a circulating marker, a membrane associated marker, or a component present within a vesicle or on a vesicle's surface. These biomarkers include without limitation a nucleic acid (e.g. RNA (mRNA, miRNA, etc.) or DNA), protein, peptide, polypeptide, antigen, lipid, carbohydrate, or proteoglycan.

PREOMICS EXHIBIT 1002
Page 1017 of 1861

[00377] The biosignature can include the presence or absence, expression level, mutational state, genetic variant state, or any modification (such as epigenetic modification, or post-translation modification) of a biomarker disclosed herein (e.g., **Tables 3** or **5**) or previously disclosed (e.g. any one or more biomarker listed in **Figs. 1, 3-60** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein). One of skill will recognize that methods of the invention can be adapted to assess one or more biomarkers disclosed herein for a disease or condition different than a disease that is conventionally associated with a given biomarker. For example, one or more biomarkers disclosed herein for condition *x* may readily be utilized in obtaining a biosignature for a different condition *y*, based on the teachings of the instant disclosure and methods of the invention. The expression level of a biomarker can be compared to a control or reference, to determine the overexpression or underexpression (or upregulation or downregulation) of a biomarker in a sample. In some embodiments, the control or reference level comprises the amount of a same biomarker, such as a miRNA, in a control sample from a subject that does not have or exhibit the condition or disease. In another embodiment, the control of reference levels comprises that of a housekeeping marker whose level is minimally affected, if at all, in different biological settings such as diseased versus non-diseased states. In yet another embodiment, the control or reference level comprises that of the level of the same marker in the same subject but in a sample taken at a different time point. Other types of controls are described herein.

[00378] Nucleic acid biomarkers include various RNA or DNA species. For example, the biomarker can be mRNA, microRNA (miRNA), small nucleolar RNAs (snoRNA), small nuclear RNAs (snRNA), ribosomal RNAs (rRNA), heterogeneous nuclear RNA (hnRNA), ribosomal RNAS (rRNA), siRNA, transfer RNAs (tRNA), or shRNA. The DNA can be double-stranded DNA, single stranded DNA, complementary DNA, or noncoding DNA. miRNAs are short ribonucleic acid (RNA) molecules which average about 22 nucleotides long. miRNAs act as post-transcriptional regulators that bind to complementary sequences in the three prime untranslated regions (3' UTRs) of target messenger RNA transcripts (mRNAs), which can result in gene silencing. One miRNA may act upon 1000s of mRNAs. miRNAs play multiple roles in negative regulation, e.g., transcript degradation and sequestering, translational suppression, and may also have a role in positive regulation, e.g., transcriptional and translational activation. By affecting gene regulation, miRNAs can influence many biologic processes. Different sets of expressed miRNAs are found in different cell types and tissues.

[00379] Biomarkers for use with the invention further include peptides, polypeptides, or proteins, which terms are used interchangeably throughout unless otherwise noted. In some embodiments, the protein biomarker comprises its modification state, truncations, mutations, expression level (such as overexpression or underexpression as compared to a reference level), and/or post-translational modifications, such as described above. In a non-limiting example, a biosignature for a disease can include a protein having a certain post-translational modification that is more prevalent in a sample associated with the disease than without.

[00380] A biosignature may include a number of the same type of biomarkers (e.g., two or more different microRNA or mRNA species) or one or more of different types of biomarkers (e.g. mRNAs, miRNAs, proteins, peptides, ligands, and antigens).

[00381] One or more biosignatures can comprise at least one biomarker selected from those listed in **Figs. 1, 3-60** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein. A specific

PREOMICS EXHIBIT 1002
Page 1018 of 1861

cell-of-origin biosignature may include one or more biomarkers. **Figs. 3-58** of International Patent Application Serial No. PCT/US201 1/03 1479 depict tables which lists a number of disease or condition specific biomarkers that can be derived and analyzed from a vesicle. The biomarker can also be CD24, midkine, hepcidin, TMPRSS2-ERG, PCA-3, PSA, EGFR, EGFRvIII, BRAF variant, MET, cKit, PDGFR, Wnt, beta-catenin, K-ras, H-ras, N-ras, Raf, N-myc, c-myc, IGFR, PI3K, Akt, BRCA1, BRCA2, PTEN, VEGFR-2, VEGFR-1, Tie-2, TEM-1, CD276, HER-2, HER-3, or HER-4. The biomarker can also be annexin V, CD63, Rab-5b, or caveolin, or a miRNA, such as let-7a; miR-15b; miR-16; miR-19b; miR-21 ; miR-26a; miR-27a; miR-92; miR-93; miR-320 or miR-20. The biomarker can also be of any gene or fragment thereof as disclosed in PCT Publication No. WO2009/100029, such as those listed in Tables 3-15 therein.

[00382] In another embodiment, a vesicle comprises a cell fragment or cellular debris derived from a rare cell, such as described in PCT Publication No. WO2006054991. One or more biomarkers, such as CD 146, CD 105, CD3 1, CD 133, CD 106, or a combination thereof, can be assessed for the vesicle. In one embodiment, a capture agent for the one or more biomarkers is used to isolate or detect a vesicle. In some embodiments, one or more of the biomarkers CD45, cytokeratin (CK) 8, CK1 8, CK19, CK20, CEA, EGFR, GUC, EpCAM, VEGF, TS, Muc-1, or a combination thereof is assessed for a vesicle. In one embodiment, a tumor-derived vesicle is CD45-, CK+ and comprises a nucleic acid, wherein the membrane vesicle has an absence of, or low expression or detection of CD45, has detectable expression of a cytokeratin (such as CK8, CK1 8, CK19, or CK20), and detectable expression of a nucleic acid.

[00383] Any number of useful biomarkers that can be assessed as part of a vesicle biosignature are disclosed throughout the application, including without limitation CD9, EphA2, EGFR, B7H3, PSM, PCSA, CD63, STEAP, CD81, ICAM1, A33, DR3, CD66e, MFG-E8, TROP-2, Mammaglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, EpCam, neurokinin receptor- 1 (NK-1 or NK-lR), NK-2, Pai-1, CD45, CD10, HER2/ERBB2, AGTR1, NPY1R, MUC1, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUC1 secreted), CA27.29 (MUC1 secreted), NMDAR1, NMDAR2, MAGEA, CTAG1B, NY-ESO-1, SPB, SPC, NSE, PGP9.5, P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, IC3b, mesothelin, SPA, AQP5, GPCR, hCEA-CAM, PTP IA-2, CABYR, TMEM21 1, ADAM28, UNC93A, MUC17, MUC2, ILlOR-beta, BCMA, HVEM/TNFRSF14, Trappin-2 Elafm, ST2/IL1 R4, TNFRF 14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNFR, or a combination thereof.

[00384] Other biomarkers useful for assessment in methods and compositions disclosed herein include those associated with conditions or physiological states as disclosed in U.S. Patent No. 6329179 and 7,625,573; U.S. Patent Publication Nos. 2002/106684, 2004/005596, 2005/0159378, 2005/0064470, 2006/1 16321, 2007/0161004, 2007/0077553, 2007/104738, 2007/02981 18, 2007/0172900, 2008/0268429, 2010/0062450, 2007/02981 18, 2009/0220944 and 2010/0196426; U.S. Patent Application Nos. 12/524,432, 12/524,398, 12/524,462; Canadian Patent CA 2453 198; and International PCT Patent Publication Nos. WO199402201 8, WO2001036601, WO2003063690, WO2003044166, WO2003076603, WO2005 121369, WO20051 18806, WO/2005/078124, WO2007126386, WO2007088537, WO2007103572, WO2009019215, WO2009021322, WO2009036236, WO2009100029, WO2009015357, WO2009155505, WO 2010/065968 and WO 2010/070276; each of which patent or application is incorporated herein by reference in their entirety. The biomarkers disclosed in these patents and applications, including vesicle biomarkers and microRNAs, can be assessed as part of a signature for characterizing a phenotype, such as providing a diagnosis, prognosis or

PREOMICS EXHIBIT 1002
Page 1019 of 1861

theranosis of a cancer or other disease. Furthermore, the methods and techniques disclosed therein can be used to assess biomarkers, including vesicle biomarkers and microRNAs.

[00385] Another group of useful biomarkers for assessment in methods and compositions disclosed herein include those associated with cancer diagnostics, prognostics and theranostics as disclosed in US Patents 6,692,916, 6,960,439, 6,964,850, 7,074,586; U.S. Patent Application Nos. 11/159,376, 11/804,175, 12/594,128, 12/5 14,686, 12/5 14,775, 12/594,675, 12/594,91 1, 12/594,679, 12/741,787, 12/3 12,390; and International PCT Patent Application Nos. PCT/US2009/049935, PCT/US2009/063 138, PCT/US2010/000037; each of which patent or application is incorporated herein by reference in their entirety. Usefule biomarkers further include those described in U.S. Patent Application Nos., 10/703,143 and US 10/701,391 for inflammatory disease; 11/529,010 for rheumatoid arthritis; 11/454,553 and 11/827,892 for multiple sclerosis; 11/897, 160 for transplant rejection; 12/524,677 for lupus; PCT/US2009/048684 for osteoarthritis; 10/742,458 for infectious disease and sepsis; 12/520,675 for sepsis; each of which patent or application is incorporated herein by reference in their entirety. The biomarkers disclosed in these patents and applications, including mRNAs, can be assessed as part of a signature for characterizing a phenotype, such as providing a diagnosis, prognosis or theranosis of a cancer or other disease. Furthermore, the methods and techniques disclosed therein can be used to assess biomarkers, including vesicle biomarkers and microRNAs.

[00386] Still other biomarkers useful for assessment in methods and compositions disclosed herein include those associated with conditions or physiological states as disclosed in Wieczorek *et al.,* Isolation and characterization of an RNA-proteolipid complex associated with the malignant state in humans, Proc Natl Acad Sci U S A. 1985 May;82(10):3455-9; Wieczorek *et al.*, Diagnostic and prognostic value of RNA-proteolipid in sera of patients with malignant disorders following therapy: first clinical evaluation of a novel tumor marker, Cancer Res. 1987 Dec 1;47(23):6407-12; Escola *et al. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes.* J. Biol. Chem. (1998) **273**:20121-27; Pileri *et al. Binding of hepatitis C virus to CD81* Science, (1998) **282**:938-41); Kopreski *et al. Detection of Tumor Messenger RNA in the Serum of Patients with Malignant Melanoma,* Clin. Cancer Res. (1999) **5**:1961-1965; Carr *et al. Circulating Membrane Vesicles in Leukemic Blood,* Cancer Research, (1985) **45**:5944-51; Weichert *et al.* Cytoplasmic CD24 expression in colorectal cancer independently correlates with shortened patient survival. Clinical Cancer Research, 2005, 11:6574-81); Iorio *et al. MicroRNA gene expression deregulation in human breast cancer.* Cancer Res (2005) **65**:7065-70; Taylor *et al. Tumour-derived exosomes and their role in cancer-associated T-cell signaling defects* British J Cancer (2005) **92**:305-1 1; Valadi *et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells* Nature Cell Biol (2007) **9**:654-59; Taylor *et al. Pregnancy-associated exosomes and their modulation of T cell signaling* J Immunol (2006) **176:** 1534-42; Koga *et al. Purification, characterization and biological significance of tumor-derived exosomes* Anticancer Res (2005) **25**:3703-08; Seligson *et al. Epithelial cell adhesion molecule (KSA) expression: pathobiology and its role as an independent predictor of survival in renal cell carcinoma* Clin Cancer Res (2004) **10**:2659-69; Clayton *et al. (Antigen-presenting cell exosomes are protected from complement-mediated lysis by expression of CD55 and CD59.* Eur J Immunol (2003) **33**:522-3 1); Simak *et al. Cell Membrane Microparticles in Blood and Blood Products: Potentially Pathogenic Agents and Diagnostic Markers* Trans Med Reviews (2006) **20**:1-26; Choi *et al. Proteomic analysis of microvesicles derived from human colorectal cancer cells* J Proteome Res (2007) **6**:4646-4655; Iero *et al. Tumour-released exosomes and*

PREOMICS EXHIBIT 1002
Page 1020 of 1861

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[00387] Still other biomarkers useful for assessment in methods and compositions disclosed herein include those associated with conditions or physiological states as disclosed in *Rajendran et al, Proc Natl Acad Sci U S A 2006;103:111 72-111 77, Taylor et al, Gynecol Oncol 2008;110:13-21, Zhou et al, Kidney Int 2008; 74:613-621, Buning et al, Immunology 2008, Prado et al. J Immunol 2008;181:1519-1525, Vella et al. (2008) Vet Immunol Immunopathol 124(3-4): 385-93, Gould et al. (2003). Proc Natl Acad Sci USA 100(19): 10592-7, Fang et al. (2007). PLoS Biol 5(6): el 58, Chen, B. J. and R. A. Lamb (2008). Virology 372(2): 221-32, Bhatnagar, S. and J. S. Schorey (2007). J Biol Chem 282(35): 25779-89, Bhatnagar et al. (2007) Blood 110(9):*

PREOMICS EXHIBIT 1002
Page 1021 of 1861

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[00388] In another aspect, the invention provides a method of assessing a cancer comprising detecting a level of one or more circulating biomarkers in a sample from a subject selected from the group consisting of CD9, HSP70, Gal3, MIS, EGFR, ER, ICB3, CD63, B7H4, MUC1, DLL4, CD81, ERB3, VEGF, BCA225, BRCA, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD3 1, cMET, MUC2 or ERB4. CD9, HSP70, Gal3, MIS, EGFR, ER, ICB3, CD63, B7H4, MUC1, DLL4, CD81, ERB3, VEGF, BCA225, BRCA, BCA200, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD3 1, cMET, MUC2 or ERB4. In another embodiment, the one or more circulating biomarkers are selected from the group consisting of CD9, EphA2, EGFR, B7H3, PSMA, PCSA, CD63, STEAP, STEAP, CD81, B7H3, STEAPl, ICAMl (CD54), PSMA, A33, DR3, CD66e, MFG-8e, EphA2, Hepsin, TMEM21 1, EphA2, TROP-2, EGFR, Mammoglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, NK-2, EpCam, NGAL, NK-1R, PSMA, 5T4, PAI-1, and CD45. In still another embodiment, the one or more circulating biomarkers are selected from the group consisting of CD9, MIS Rii, ER, CD63, MUC1, HER3, STAT3, VEGFA, BCA, CA125, CD24, EPCAM, and ERB B4. Any number of useful biomarkers can be assessed from these groups, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the one or more biomarkers are one or more of Gal3, BCA200, OPN and NCAM, e.g., Gal3 and BCA200, OPN and NCAM, or all four. Assessing the cancer may comprise diagnosing, prognosing or theranosing the cancer. The cancer can be a breast cancer. The markers can be associated with a vesicle or vesicle population. For example, the one or more circulating biomarker can be a vesicle surface antigen or vesicle payload. Vesicle surface antigens can further be used as capture antigens, detector antigens, or both.

[00389] The invention further provides a method for predicting a response to a therapeutic agent comprising detecting a level of one or more circulating biomarkers in a sample from a subject selected from the group consisting of CD9, HSP70, Gal3, MIS, EGFR, ER, ICB3, CD63, B7H4, MUC1, DLL4, CD81, ERB3, VEGF, BCA225, BRCA, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD3 1, cMET, MUC2 or ERB4. Biomarkers can also be selected from the group consisting of CD9, EphA2, EGFR, B7H3, PSMA, PCSA, CD63, STEAP, STEAP, CD81, B7H3, STEAPl, ICAMl (CD54), PSMA, A33, DR3, CD66e, MFG-8e, EphA2, Hepsin, TMEM21 1, EphA2, TROP-2, EGFR, Mammoglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, NK-2, EpCam, NGAL, NK-1R, PSMA, 5T4, PAI-1, and CD45. In still another embodiment, the one or more circulating biomarkers are selected from the group consisting of CD9, MIS Rii, ER, CD63, MUC1, HER3,

PREOMICS EXHIBIT 1002
Page 1022 of 1861

STAT3, VEGFA, BCA, CA125, CD24, EPCAM, and ERB B4. Any number of useful biomarkers can be assessed from these groups, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the one or more biomarkers are one or more of Gal3, BCA200, OPN and NCAM, e.g., Gal3 and BCA200, OPN and NCAM, or all four. The therapeutic agent can be a therapeutic agent for treating cancer. The cancer can be a breast cancer. The markers can be associated with a vesicle or vesicle population. For example, the one or more circulating biomarker can be a vesicle surface antigen or vesicle payload. Vesicle surface antigens can further be used as capture antigens, detector antigens, or both.

[00390] Various methods or platforms can be used to assess or detect biomarkers identified herein. Examples of such methods or platforms include but are not limited to using an antibody array, microbeads, or other method disclosed herein or known in the art. For example, a capture antibody or aptamer to the one or more biomarkers can be bound to the array or bead. The captured vesicles can then be detected using a detectable agent. In some embodiments, captured vesicles are detected using an agent, e.g., an antibody or aptamer, that recognizes general vesicle biomarkers that detect the overall population of vesicles, such as a tetraspanin or MFG-E8. These can include tetraspanins such as CD9, CD63 and/or CD81 . In other embodiments, the captured vesicles are detected using markers specific for vesicle origin, e.g., a type of tissue or organ. In some embodiments, the captured vesicles are detected using CD3 1, a marker for cells or vesicles of endothelial origin. As desired, the biomarkers used for capture can also be used for detection, and vice versa.

[00391] Methods of the invention can be used to assess various diseases or conditions, where biomarkers correspond to various such diseases or conditions. For example, methods of the invention are applied to assess one or more cancers, such as those disclosed herein, wherein a method comprises detecting a level of one or more circulating biomarker in a sample from a subject selected from the group consisting of 5T4 (trophoblast), ADAM 10, AGER/RAGE, APC, APP (β-amyloid), ASPH (A-10), B7H3 (CD276), BACE1, BAI3, BRCA1, BDNF, BIRC2, C1GALT1, CA125 (MUC16), Calmodulin 1, CCL2 (MCP-1), CD9, CD10, CD127 (IL7R), CD174, CD24, CD44, CD63, CD81, CEA, CRMP-2, CXCR3, CXCR4, CXCR6, CYFRA 21, derlin 1, DLL4, DPP6, E-CAD, EpCaM, EphA2 (H-77), ER(1) ESR1 a, ER(2) ESR2 β, Erb B4, Erbb2, erb3 (Erb-B3), PA2G4, FRT (FLT1), Gal3, GPR30 (G-coupled ER1), HAP1, HER3, HSP-27, HSP70, IC3b, IL8, insig, junction plakoglobin, Keratin 15, KRAS, Mammaglobin, MARTI, MCT2, MFGE8, MMP9, MRP8, Mucl, MUC17, MUC2, NCAM, NG2 (CSPG4), Ngal, NHE-3, NT5E (CD73), ODCl, OPG, OPN, p53, PARK7, PCSA, PGP9.5 (PARK5), PR(B), PSA, PSMA, RAGE, STXBP4, Survivin, TFF3 (secreted), TIMP1, TIMP2, TMEM21 1, TRAF4 (scaffolding), TRAIL-R2 (death Receptor 5), TrkB, Tsg 101, UNC93a, VEGF A, VEGFR2, YB-1, VEGFR1, GCDPF-15 (PIP), BigH3 (TGFbl -induced protein), 5HT2B (serotonin receptor 2B), BRCA2, BACE 1, CDHl-cadherin. The methods can comprise detecting protein, RNA or DNA of the specified target biomarker. The one or more marker can be assessed directly from a biological fluid, such as those fluids disclosed herein, or can be assessed for its association with a vesicle, e.g., as a vesicle surface antigen or as vesicle payload (e.g., soluble protein, mRNA or DNA). A particular biosignature determined using methods and compositions of the invention can comprise any number of useful biomarkers, e.g., a biosignature can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more different biomarkers (or in some cases different molecules of the same biomarkers, such protein and nucleic acid). Vesicle surface antigens can also be used as capture antigens, detector antigens, or both, as disclosed herein or in applications incorporated by reference.

PREOMICS EXHIBIT 1002
Page 1023 of 1861

[00392] Methods and compositions of the invention are applied to assess various aspects of a cancer, including identifying different informative aspects of a cancer, e.g., identifying a biosignature that is indicative of metastasis, angiogenesis, or classifying different stages, classes or subclasses of the same tumor or tumor lineage.

[00393] Furthermore, methods of the invention comprise determining if a disease or condition affects immunomodulation in a subject. For example, the one or more circulating biomarker for immunomodulation can be one or more of CD45, FasL, CTLA4, CD80 and CD83. The one or more circulating biomarker for metastatis can be one or more of Mucl, CD147, TIMP1, TIMP2, MMP7, and MMP9. The one or more circulating biomarker for angiogenesis can be one or more of HIF2a, Tie2, Angl, DLL4 and VEGFR2. Any number of useful biomarkers can be assessed from the groups, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. The cancer can be a breast cancer. The markers can be associated with a vesicle or vesicle population. For example, the one or more circulating biomarker can be a vesicle surface antigen or vesicle payload. Vesicle surface antigens can further be used as capture antigens, detector antigens, or both.

[00394] A biosignature can comprise DLL4 or cMET. Delta-like 4 (DLL4) is a Notch-ligand and is up-regulated during angiogenesis. cMET (also referred to as c-Met, MET, or MNNG HOS Transforming gene) is a proto-oncogene that encodes a membrane receptor tyrosine kinase whose ligand is hepatocyte growth factor (HGF). The MET protein is sometimes referred to as the hepatocyte growth factor receptor (HGFR). MET is normally expressed on epithelial cells, and improper activation can trigger tumor growth, angiogenesis and metastasis. DLL4 and cMET can be used as biomarkers to detect a vesicle population.

[00395] Biomarkers that can be derived and analyzed from a vesicle include miRNA (miR), miRNA*nonsense (miR*), and other RNAs (including, but not limited to, mRNA, preRNA, priRNA, hnRNA, snRNA, siRNA, shRNA). A miRNA biomarker can include not only its miRNA and microRNA* nonsense, but its precursor molecules: pri-microRNAs (pri-miRs) and pre-microRNAs (pre-miRs). The sequence of a miRNA can be obtained from publicly available databases such as http://www.mirbase.org/, http://www.microrna.org/, or any others available. Unless noted, the terms miR, miRNA and microRNA are used interchangeably throughout unless noted. In some embodiments, the methods of the invention comprise isolating vesicles, and assessing the miRNA payload within the isolated vesicles. The biomarker can also be a nucleic acid molecule (e.g. DNA), protein, or peptide. The presence or absence, expression level, mutations (for example genetic mutations, such as deletions, translocations, duplications, nucleotide or amino acid substitutions, and the like) can be determined for the biomarker. Any epigenetic modulation or copy number variation of a biomarker can also be analyzed.

[00396] The one or more biomarkers analyzed can be indicative of a particular tissue or cell of origin, disease, or physiological state. Furthermore, the presence, absence or expression level of one or more of the biomarkers described herein can be correlated to a phenotype of a subject, including a disease, condition, prognosis or drug efficacy. The specific biomarker and biosignature set forth below constitute non-inclusive examples for each of the diseases, condition comparisons, conditions, and/or physiological states. Furthermore, the one or more biomarker assessed for a phenotype can be a cell-of-origin specific vesicle.

[00397] The one or more miRNAs used to characterize a phenotype may be selected from those disclosed in PCT Publication No. WO2009/036236. For example, one or more miRNAs listed in Tables I-VI (Figures 6-1 1) therein can be used to characterize colon adenocarcinoma, colorectal cancer, prostate cancer, lung cancer, breast cancer, b- cell lymphoma, pancreatic cancer, diffuse large BCL cancer, CLL, bladder cancer, renal cancer,

PREOMICS EXHIBIT 1002
Page 1024 of 1861

hypoxia-tumor, uterine leiomyomas, ovarian cancer, hepatitis C virus-associated hepatocellular carcinoma, ALL, Alzheimer's disease, myelofibrosis, myelofibrosis, polycythemia vera, thrombocythemia, HIV, or HIV-I latency, as further described herein.

[00398] The one or more miRNAs can be detected in a vesicle. The one or more miRNAs can be miR-223, miR-484, miR- 191, miR-146a, miR-016, miR-026a, miR-222, miR-024, miR-126, and miR-32. One or more miRNAs can also be detected in PBMC. The one or more miRNAs can be miR-223, miR-150, miR-146b, miR-016, miR-484, miR-146a, miR-191, miR-026a, miR-019b, or miR-020a. The one or more miRNAs can be used to characterize a particular disease or condition. For example, for the disease bladder cancer, one or more miRNAs can be detected, such as miR-223, miR-26b, miR-221, miR-103-1, miR-1 85, miR-23b, miR-203, miR-17-5p, miR-23a, miR-205 or any combination thereof. The one or more miRNAs may be upregulated or overexpressed.

[00399] In some embodiments, the one or more miRNAs is used to characterize hypoxia-tumor. The one or more miRNA may be miR-23, miR-24, miR-26, miR-27, miR-103, miR- 107, miR-1 81, miR-210, or miR-213, and may be upregulated. One or more miRNAs can also be used to characterize uterine leiomyomas. For example, the one or more miRNAs used to characterize a uterine leiomyoma may be a let-7 family member, miR-21, miR-23b, miR-29b, or miR-197. The miRNA can be upregulated.

[00400] Myelofibrosis can also be characterized by one or more miRNAs, such as miR-190, which can be upregulated; miR-3 1, miR-150 and miR-95, which can be downregulated, or any combination thereof. Furthermore, myelofibrosis, polycythemia vera or thrombocythemia can also be characterized by detecting one or more miRNAs, such as, but not limited to, miR-34a, miR-342, miR-326, miR- 105, miR-149, miR- 147, or any combination thereof. The one or more miRNAs may be downregulated.

[00401] Other examples of phenotypes that can be characterized by assessing a vesicle for one or more biomarkers are futher described herein.

[00402] The one or more biomarkers can be detected using a probe. A probe can comprise an oligonucleotide, such as DNA or RNA, an aptamer, monoclonal antibody, polyclonal antibody, Fabs, Fab', single chain antibody, synthetic antibody, peptoid, zDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), lectin, synthetic or naturally occurring chemical compound (including but not limited to a drug or labeling reagent), dendrimer, or a combination thereof. The probe can be directly detected, for example by being directly labeled, or be indirectly detected, such as through a labeling reagent. The probe can selectively recognize a biomarker. For example, a probe that is an oligonucleotide can selectively hybridize to a miRNA biomarker.

[00403] In aspects, the invention provides for the diagnosis, theranosis, prognosis, disease stratification, disease staging, treatment monitoring or predicting responder / non-responder status of a disease or disorder in a subject. The invention comprises assessing vesicles from a subject, including assessing biomarkers present on the vesicles and/or assessing payload within the vesicles, such as protein, nucleic acid or other biological molecules. Any appropriate biomarker that can be assessed using a vesicle and that relates to a disease or disorder can be used the carry out the methods of the invention. Furthermore, any appropriate technique to assess a vesicle as described herein can be used. Exemplary biomarkers for specific diseases that can be assessed according to the methods of the invention include the biomarkers described in International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein.

PREOMICS EXHIBIT 1002
Page 1025 of 1861

[00404] Any of the types of biomarkers or specific biomarkers described herein can be assessed to identify a biosignature or to identify a candidate biosignature. Exemplary biomarkers include without limitation those in **Table 5.** The markers in the table can be used for capture and/or detection of vesicles for characterizing phenotypes as disclosed herein. In some cases, multiple capture and/or detectors are used to enhance the characterization. The markers can be detected as protein or as mRNA, which can be circulating freely or in a complex with other biological molecules. The markers can be detected as vesicle surface antigens or and vesicle payload. The "Illustrative Class" indicates indications for which the markers are known markers. Those of skill will appreciate that the markers can also be used in alternate settings in certain instances. For example, a marker which can be used to characterize one type disease may also be used to characterize another disease as appropriate. Consider a non-limiting example of a tumor marker which can be used as a biomarker for tumors from various lineages.

Table 5: Illustrative Vesicle Associated Biomarkers

Illustrative Class	Biomarkers
Drug associated targets and prognostic markers	ABCC1, ABCG2, ACE2, ADA, ADH1C, ADH4, AGT, AR, AREG, ASNS, BCL2, BCRP, BDCA1, beta III tubulin, BIRC5, B-RAF, BRCA1, BRCA2, CA2, caveolin, CD20, CD25, CD33, CD52, CDA, CDKN2A, CDKN1A, CDKN1B, CDK2, CDW52, CES2, CK 14, CK 17, CK 5/6, c-KIT, c-Met, c-Myc, COX-2, Cyclin D1, DCK, DHFR, DNMT1, DNMT3A, DNMT3B, E-Cadherin, ECGF1, EGFR, EML4-ALK fusion, EPHA2, Epiregulin, ER, ERBR2, ERCC1, ERCC3, EREG, ESR1, FLT1, folate receptor, FOLR1, FOLR2, FSHB, FSHPRH1, FSHR, FYN, GART, GNA11, GNAQ, GNRH1, GNRHR1, GSTP1, HCK, HDAC1, hENT-1, Her2/Neu, HGF, HIF1A, HIG1, HSP90, HSP90AA1, HSPCA, IGF-1R, IGFRBP, IGFRBP3, IGFRBP4, IGFRBP5, IL13RA1, IL2RA, KDR, Ki67, KIT, K-RAS, LCK, LTB, Lymphotoxin Beta Receptor, LYN, MET, MGMT, MLH1, MMR, MRP1, MS4A1, MSH2, MSH5, Myc, NFKB1, NFKB2, NFKBIA, NRAS, ODC1, OGFR, p16, p21, p27, p53, p95, PARP-1, PDGFC, PDGFR, PDGFRA, PDGFRB, PGP, PGR, PI3K, POLA, POLA1, PPARG, PPARGC1, PR, PTEN, PTGS2, PTPN12, RAF1, RARA, ROS1, RRM1, RRM2, RRM2B, RXRB, RXRG, SIK2, SPARC, SRC, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, Survivin, TK1, TLE3, TNF, TOP1, TOP2A, TOP2B, TS, TUBB3, TXN, TXNRD1, TYMS, VDR, VEGF, VEGFA, VEGFC, VHL, YES1, ZAP70
Cancer treatment associated markers	AR, AREG (Amphiregulin), BRAF, BRCA1, cKIT, cMET, EGFR, EGFR w/T790M, EML4-ALK, ER, ERBB3, ERBB4, ERCC1, EREG, GNA11, GNAQ, hENT-1, Her2, Her2 Exon 20 insert, IGF1R, Ki67, KRAS, MGMT, MGMT methylation, MSH2, MSI, NRAS, PGP (MDR1), PIK3CA, PR, PTEN, ROS1, ROS1 translocation, RRM1, SPARC, TLE3, TOPO1, TOPO2A, TS, TUBB3, VEGFR2
Cancer treatment associated markers	AR, AREG, BRAF, BRCA1, cKIT, cMET, EGFR, EGFR w/T790M, EML4-ALK, ER, ERBB3, ERBB4, ERCC1, EREG, GNA11, GNAQ, Her2, Her2 Exon 20 insert, IGFR1, Ki67, KRAS, MGMT-Me, MSH2, MSI, NRAS, PGP (MDR-1), PIK3CA, PR, PTEN, ROS1 translocation, RRM1, SPARC, TLE3, TOPO1, TOPO2A, TS, TUBB3, VEGFR2
Colon cancer treatment associated markers	AREG, BRAF, EGFR, EML4-ALK, ERCC1, EREG, KRAS, MSI, NRAS, PIK3CA, PTEN, TS, VEGFR2
Colon cancer treatment associated markers	AREG, BRAF, EGFR, EML4-ALK, ERCC1, EREG, KRAS, MSI, NRAS, PIK3CA, PTEN, TS, VEGFR2
Melanoma treatment associated markers	BRAF, cKIT, ERBB3, ERBB4, ERCC1, GNA11, GNAQ, MGMT, MGMT methylation, NRAS, PIK3CA, TUBB3, VEGFR2
Melanoma treatment associated markers	BRAF, cKIT, ERBB3, ERBB4, ERCC1, GNA11, GNAQ, MGMT-Me, NRAS, PIK3CA, TUBB3, VEGFR2
Ovarian cancer treatment associated	BRCA1, cMET, EML4-ALK, ER, ERBB3, ERCC1, hENT-1, HER2, IGF1R, PGP(MDR1), PIK3CA, PR, PTEN, RRM1, TLE3, TOPO1, TOPO2A, TS

PREOMICS EXHIBIT 1002
Page 1026 of 1861

markers	
Ovarian cancer treatment associated markers	BRCA1, cMET, EML4-ALK (translocation), ER, ERBB3, ERCC1, HER2, PIK3CA, PR, PTEN, RRMl, TLE3, TS
Breast cancer treatment associated markers	BRAF, BRCA1, EGFR, EGFR T790M, EML4-ALK, ER, ERBB3, ERCC1, HER2, Ki67, PGP (MDR1), PIK3CA, PR, PTEN, ROS1, ROSl translocation, RRMl, TLE3, TOPOl, TOP02A, TS
Breast cancer treatment associated markers	BRAF, BRCA1, EGFR w/T790M, EML4-ALK, ER, ERBB3, ERCC1, HER2, Ki67, KRAS, PIK3CA, PR, PTEN, ROSl translocation, RRMl, TLE3, TOPOl, TOP02A, TS
NSCLC cancer treatment associated markers	BRAF, BRCA1, cMET, EGFR, EGFR w/T790M, EML4-ALK, ERCC1, Her2 Exon 20 insert, KRAS, MSH2, PIK3CA, PTEN, ROSl (trans), RRMl, TLE3, TS, VEGFR2
NSCLC cancer treatment associated markers	BRAF, cMET, EGFR, EGFR w/T790M, EML4-ALK, ERCC1, Her2 Exon 20 insert, KRAS, MSH2, PIK3CA, PTEN, ROSl translocation, RRMl, TLE3, TS
Cancer/Angio	Erb 2, Erb 3, Erb 4, UNC93a, B7H3, MUC1, MUC2, MUC16, MUC17, 5T4, RAGE, VEGF A, VEGFR2, FLT1, DLL4, Epcam
Tissue (Breast)	BIG H3, GCDFP-15, PR(B), GPR 30, CYFRA 21, BRCA 1, BRCA 2, ESR 1, ESR2
Tissue (Prostate)	PSMA, PCSA, PSCA, PSA, TMPRSS2
Inflammation/Immune	MFG-E8, IFNAR, CD40, CD80, MICB, HLA-DRb, IL-17-Ra
Common vesicle markers	HSPA8, CD63, Actb, GAPDH, CD9, CD81, ANXA2, HSP90AA1, ENOl, YWHAZ, PDCD6IP, CFL1, SDCBP, PKN2, MSN, MFGE8, EZR, YWHAG, PGK1, EEF1A1, PPIA, GLC1F, GK, ANXA6, ANXA1, ALDOA, ACTG1, TPI1, LAMP2, HSP90AB1, DPP4, YWHAB, TSG101, PFN1, LDHB, HSPA1B, HSPAIA, GSTPl, GNAI2, GDI2, CLTC, ANXA5, YWHAQ, TUBAIA, THBSl, PRDX1, LDHA, LAMP1, CLU, CD86
Common vesicle membrane markers	CD63, GAPDH, CD9, CD81, ANXA2, ENOl, SDCBP, MSN, MFGE8, EZR, GK, ANXA1, LAMP2, DPP4, TSG101, HSPAIA, GDI2, CLTC, LAMP1, CD86, ANPEP, TFRC, SLC3A2, RDX, RAP IB, RAB5C, RAB5B, MYH9, ICAM1, FN1, RAB1 1B, PIGR, LGALS3, ITGB1, EHD1, CLIC1, ATP1A1, ARF1, RAP1A, P4HB, MUC1, KRT10, HLA-A, FLOT1, CD59, Clorf58, BASP1, TACSTD1, STOM
Common vesicle markers	MHC class I, MHC class II, Integrins, Alpha 4 beta 1, Alpha M beta 2, Beta 2, ICAM1/CD54, P-selection, Dipeptidylpeptidase IV/CD26, Aminopeptidase n/CD13, CD15 1, CD53, CD37, CD82, CD81, CD9, CD63, Hsp70, Hsp84/90 Actin, Actin-binding proteins, Tubulin, Annexin I, Annexin II, Annexin IV, Annexin V, Annexin VI, RAB7/RAP 1B/RADGDI, Gi2alpha/14-3-3, CBL/LCK, CD63, GAPDH, CD9, CD81, ANXA2, ENOl, SDCBP, MSN, MFGE8, EZR, GK, ANXAl, LAMP2, DPP4, TSG101, HSPAIA, GDI2, CLTC, LAMP1, Cd86, ANPEP, TFRC, SLC3A2, RDX, RAP IB, RAB5C, RAB5B, MYH9, ICAM1, FN1, RAB1 1B, PIGR, LGALS3, ITGB1, EHD1, CLIC1, ATP1A1, ARF1, RAP1A, P4HB, MUC1, KRT10, HLA-A, FLOT1, CD59, Clorf58, BASP1, TACSTD1, STOM
Vesicle markers	A33, a33 nl5, AFP, ALA, ALIX, ALP, AnnexinV, APC, ASCA, ASPH (246-260), ASPH (666-680), ASPH (A-10), ASPH (D01P), ASPH (D03), ASPH (G-20), ASPH (H-300), AURKA, AURKB, B7H3, B7H4, BCA-225, BCNP, BDNF, BRCA, CA125 (MUC16), CA-19-9, C-Bir, CD1 .1, CD10, CD174 (Lewis y), CD24, CD44, CD46, CD59 (MEM-43), CD63, CD66e CEA, CD73, CD81, CD9, CDA, CDAC1 la2, CEA, C-Erb2, C-erbB2, CRMP-2, CRP, CXCL12, CYFRA21-1, DLL4, DR3, EGFR, Epcam, EphA2, EphA2 (H-77), ER, ErbB4, EZH2, FASL, FRT, FRT c.123, GDF15, GPCR, GPR30, Gro-alpha, HAP, HBD 1, HBD2, HER 3 (ErbB3), HSP, HSP70, hVEGFR2, iC3b, IL 6 Unc, IL-1B, IL6 Unc, IL6R, IL8, IL-8, INSIG-2, KLK2, LICAM, LAMN, LDH, MACC-1, MAPK4, MART-1, MCP-1, M-CSF, MFG-E8, MICl, MIF, MIS RII, MMG, MMP26, MMP7, MMP9, MS4A1, MUC1, MUC1 seql, MUC1 seql lA, MUC17, MUC2, Ncam, NGAL, NPGP/NPFF2, OPG, OPN, p53, p53, PA2G4, PBP, PCSA, PDGFRB, PGP9.5, PIM1, PR (B), PRL, PSA, PSMA, PSME3, PTEN, R5-

PREOMICS EXHIBIT 1002
Page 1027 of 1861

	CD9 Tube 1, Reg IV, RUNX2, SCRNl, seprase, SERPINB3, SPARC, SPB, SPDEF, SRVN, STAT 3, STEAP1, TF (FL-295), TFF3, TGM2, TIMP-1, TIMP1, TIMP2, TMEM21 1, TMPRSS2, TNF-alpha, Trail-R2, Trail-R4, TrKB, TROP2, Tsg 101, TWEAK, UNC93A, VEGF A, YPSMA-1
Vesicle markers	NSE, TRIM29, CD63, CD15 1, ASPH, LAMP2, TSPAN1, SNAIL, CD45, CKS1, NSE, FSHR, OPN, FTH1, PGP9, ANNEXIN 1, SPD, CD81, EPCAM, PTH1R, CEA, CYTO 7, CCL2, SPA, KRAS, TWIST 1, AURKB, MMP9, P27, MMP1, HLA, HIF, CEACAM, CENPH, BTUB, INTO b4, EGFR, NACC1, CYTO 18, NAP2, CYTO 19, ANNEXIN V, TGM2, ERB2, BRCA1, B7H3, SFTPC, PNT, NCAM, MS4A1, P53, INGA3, MUC2, SPA, OPN, CD63, CD9, MUCl, UNCR3, PAN ADH, HCG, TIMP, PSMA, GPCR, RACK1, PSCA, VEGF, BMP2, CD81, CRP, PRO GRP, B7H3, MUCl, M2PK, CD9, PCSA, PSMA
Vesicle markers	TFF3, MS4A1, EphA2, GAL3, EGFR, N-gal, PCSA, CD63, MUCl, TGM2, CD81, DR3, MACC-1, TrKB, CD24, TIMP-1, A33, CD66 CEA, PRL, MMP9, MMP7, TMEM21 1, SCRNl, TROP2, TWEAK, CDACC1, UNC93A, APC, C-Erb, CD10, BDNF, FRT, GPR30, P53, SPR, OPN, MUC2, GRO-1, tsg 101, GDF15
Vesicle markers	CD9, Erb2, Erb4, CD81, Erb3, MUCl 6, CD63, DLL4, HLA-Drpe, B7H3, IFNAR, 5T4, PCSA, MICB, PSMA, MFG-E8, Mucl, PSA, Muc2, Unc93a, VEGFR2, EpCAM, VEGF A, TMPRSS2, RAGE, PSCA, CD40, Mucl7, IL-17-RA, CD80
Benign Prostate Hyperplasia (BPH)	BCMA, CEACAM-1, HVEM, IL-1 R4, IL-10 Rb, Trappin-2, p53, hsa-miR-329, hsa-miR-30a, hsa-miR-335, hsa-miR-152, hsa-miR-151-5p, hsa-miR-200a, hsa-miR-145, hsa-miR-29a, hsa-miR-106b, hsa-miR-595, hsa-miR-142-5p, hsa-miR-99a, hsa-miR-20b, hsa-miR-373, hsa-miR-502-5p, hsa-miR-29b, hsa-miR-142-3p, hsa-miR-663, hsa-miR-423-5p, hsa-miR-15a, hsa-miR-888, hsa-miR-361-3p, hsa-miR-365, hsa-miR-lOb, hsa-miR-199a-3p, hsa-miR-1 81a, hsa-miR-19a, hsa-miR-125b, hsa-miR-760, hsa-miR-7a, hsa-miR-671-5p, hsa-miR-7c, hsa-miR-1979, hsa-miR-103
Metastatic Prostate Cancer	hsa-miR-100, hsa-miR-1236, hsa-miR-1296, hsa-miR-141, hsa-miR-146b-5p, hsa-miR-17*, hsa-miR-1 81a, hsa-miR-200b, hsa-miR-20a*, hsa-miR-23a*, hsa-miR-33 1-3p, hsa-miR-375, hsa-miR-452, hsa-miR-572, hsa-miR-574-3p, hsa-miR-577, hsa-miR-582-3p, hsa-miR-937, miR-lOa, miR-134, miR-141, miR-200b, miR-30a, miR-32, miR-375, miR-495, miR-564, miR-570, miR-574-3p, miR-885-3p
Metastatic Prostate Cancer	hsa-miR-200b, hsa-miR-375, hsa-miR-141, hsa-miR-33 1-3p, hsa-miR- 181a, hsa-miR-574-3p
Prostate Cancer	hsa-miR-574-3p, hsa-miR-141, hsa-miR-432, hsa-miR-326, hsa-miR-21 10, hsa-miR-1 81a-2*, hsa-miR-107, hsa-miR-301a, hsa-miR-484, hsa-miR-625*
Metastatic Prostate Cancer	hsa-miR-582-3p, hsa-miR-20a*, hsa-miR-375, hsa-miR-200b, hsa-miR-379, hsa-miR-572, hsa-miR-5 13a-5p, hsa-miR-577, hsa-miR-23a*, hsa-miR-1236, hsa-miR-609, hsa-miR-17*, hsa-miR-130b, hsa-miR-619, hsa-miR-624*, hsa-miR-198
Metastatic Prostate Cancer	FOX01A, SOX9, CLNS1A, PTGDS, XPOl, LETMD1, RAD23B, ABCC3, APC, CHES1, EDNRA, FRZB, HSPG2, TMPRSS2_ETV1 fusion
Prostate Cancer	hsa-let-7b, hsa-miR-107, hsa-miR-1205, hsa-miR-1270, hsa-miR-130b, hsa-miR-141, hsa-miR-143, hsa-miR-148b*, hsa-miR-150, hsa-miR-154*, hsa-miR-1 81a*, hsa-miR-181a-2*, hsa-miR-1 8a*, hsa-miR- 19b- 1*, hsa-miR-204, hsa-miR-21 10, hsa-miR-215, hsa-miR-217, hsa-miR-219-2-3p, hsa-miR-23b*, hsa-miR-299-5p, hsa-miR-301a, hsa-miR-301a, hsa-miR-326, hsa-miR-33 1-3p, hsa-miR-365*, hsa-miR-373*, hsa-miR-424, hsa-miR-424*, hsa-miR-432, hsa-miR-450a, hsa-miR-451, hsa-miR-484, hsa-miR-497, hsa-miR-5 17*, hsa-miR-5 17a, hsa-miR-5 18f, hsa-miR-574-3p, hsa-miR-595, hsa-miR-617, hsa-miR-625*, hsa-miR-628-5p, hsa-miR-629, hsa-miR-634, hsa-miR-769-5p, hsa-miR-93, hsa-miR-96
Prostate Cancer	CD9, PSMA, PCSA, CD63, CD81, B7H3, IL 6, OPG-13, IL6R, PA2G4, EZH2, RUNX2, SERPINB3, EpCam
Prostate Cancer	A33, a33 nl5, AFP, ALA, ALIX, ALP, AnnexinV, APC, ASCA, ASPH (246-260), ASPH (666-680), ASPH (A-10), ASPH (D01P), ASPH (D03), ASPH (G-20), ASPH (H-300), AURKA, AURKB, B7H3, B7H4, BCA-225, BCNP, BDNF, BRCA, CA125 (MUCl 6), CA-19-9, C-Bir, CD1 .1, CD10, CD174 (Lewis y), CD24, CD44, CD46, CD59 (MEM-43), CD63, CD66e CEA, CD73, CD81, CD9, CDA, CDAC1 la2, CEA, C-Erb2, C-erbB2, CRMP-2, CRP, CXCL12,

PREOMICS EXHIBIT 1002
Page 1028 of 1861

	CYFRA21-1, DLL4, DR3, EGFR, Epcam, EphA2, EphA2 (H-77), ER, ErbB4, EZH2, FASL, FRT, FRT c.f23, GDF15, GPCR, GPR30, Gro-alpha, HAP, HBD 1, HBD2, HER 3 (ErbB3), HSP, HSP70, hVEGFR2, iC3b, IL 6 Unc, IL-1B, IL6 Unc, IL6R, IL8, IL-8, INSIG-2, KLK2, LICAM, LAMN, LDH, MACC-1, MAPK4, MART-1, MCP-1, M-CSF, MFG-E8, MICl, MIF, MIS RII, MMG, MMP26, MMP7, MMP9, MS4A1, MUC1, MUC1 seql, MUC1 seql lA, MUC17, MUC2, Ncam, NGAL, NPGP/NPFF2, OPG, OPN, p53, p53, PA2G4, PBP, PCSA, PDGFRB, PGP9.5, PIM1, PR (B), PRL, PSA, PSMA, PSME3, PTEN, R5-CD9 Tube 1, Reg IV, RUNX2, SCRNl, seprase, SERPINB3, SPARC, SPB, SPDEF, SRVN, STAT 3, STEAP1, TF (FL-295), TFF3, TGM2, TIMP-1, TIMP1, TIMP2, TMEM21 1, TMPRSS2, TNF-alpha, Trail-R2, Trail-R4, TrKB, TROP2, Tsg 101, TWEAK, UNC93A, VEGF A, YPSMA-1
Prostate Cancer Vesicle Markers	5T4, ACTG1, ADAM 10, ADAM 15, ALDOA, ANXA2, ANXA6, APOA1, ATP1A1, BASP1, Clorf58, C20orfl l4, C8B, CAPZA1, CAV1, CD15 1, CD2AP, CD59, CD9, CD9, CFL1, CFP, CHMP4B, CLTC, COTL1, CTNND1, CTSB, CTSZ, CYCS, DPP4, EEF1A1, EHD1, ENOl, F11R, F2, F5, FAM125A, FNBP1L, FOLH1, GAPDH, GLB1, GPX3, HIST1H1C, HIST1H2AB, HSP90AB1, HSPA1B, HSPA8, IGSF8, ITGB1, ITIH3, JUP, LDHA, LDHB, LUM, LYZ, MFGE8, MGAM, MMP9, MYH2, MYL6B, NME1, NME2, PABPC1, PABPC4, PACSIN2, PCBP2, PDCD6IP, PRDX2, PSA, PSMA, PSMAl, PSMA2, PSMA4, PSMA6, PSMA7, PSMBl, PSMB2, PSMB3, PSMB4, PSMB5, PSMB6, PSMB8, PTGFRN, RPS27A, SDCBP, SERINC5, SH3GL1, SLC3A2, SMPDL3B, SNX9, TACSTD1, TCN2, THBS 1, TPI1, TSG101, TUBB, VDAC2, VPS37B, YWHAG, YWHAQ, YWHAZ
Prostate Cancer Vesicle Markers	FLNA, DCRN, HER 3 (ErbB3), VCAN, CD9, GAL3, CDADC1, GM-CSF, EGFR, RANK, CSA, PSMA, ChickenlgY, B7H3, PCSA, CD63, CD3, MUC1, TGM2, CD81, S100-A4, MFG-E8, Integrin, NK-2R(C-21), PSA, CD24, TIMP-1, IL6 Unc, PBP, PIM1, CA-19-9, Trail-R4, MMP9, PRL, EphA2, TWEAK, NY-ESO-1, Mammaglobin, UNC93A, A33, AURKB, CD41, XAGE-1, SPDEF, AMACR, seprase/FAP, NGAL, CXCL12, FRT, CD66e CEA, SIM2 (C-15), C-Bir, STEAP, PSIP1/LEDGF, MUC17, hVEGFR2, ERG, MUC2, ADAM 10, ASPH (A-10), CA125, Gro-alpha, Tsg 101, SSX2, Trail-R4
Prostate Cancer Vesicle Markers	NT5E (CD73), A33, ABL2, ADAM 10, AFP, ALA, ALIX, ALPL, AMACR, Apo J/CLU, ASCA, ASPH (A-10), ASPH (DOIP), AURKB, B7H3, B7H4, BCNP, BDNF, CA125 (MUC16), CA-19-9, C-Bir (Flagellin), CD10, CD15 1, CD24, CD3, CD41, CD44, CD46, CD59(MEM-43), CD63, CD66e CEA, CD81, CD9, CDA, CDADC1, C-erbB2, CRMP-2, CRP, CSA, CXCL12, CXCR3, CYFRA21-1, DCRN, DDX-1, DLL4, EGFR, EpCAM, EphA2, ERG, EZH2, FASL, FLNA, FRT, GAL3, GATA2, GM-CSF, Gro-alpha, HAP, HER3 (ErbB3), HSP70, HSPB1, hVEGFR2, iC3b, IL-1B, IL6 R, IL6 Unc, IL7 R alpha/CD127, IL8, INSIG-2, Integrin, KLK2, Label, LAMN, Mammaglobin, M-CSF, MFG-E8, MIF, MIS RII, MMP7, MMP9, MS4A1, MUC1, MUC17, MUC2, Ncam, NDUFB7, NGAL, NK-2R(C-21), NY-ESO-1, p53, PBP, PCSA, PDGFRB, PIM1, PRL, PSA, PSIP1/LEDGF, PSMA, RAGE, RANK, Reg IV, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2 (C-15), SPARC, SPC, SPDEF, SPP1, SSX2, SSX4, STEAP, STEAP4, TFF3, TGM2, TIMP-1, TMEM21 1, Trail-R2, Trail-R4, TrKB (poly), Trop2, Tsg 101, TWEAK, UNC93A, VCAN, VEGF A, wnt-5a(C-16), XAGE, XAGE-1
Prostate Cancer Treatment	hsa-miR-1974, hsa-miR-27b, hsa-miR-103, hsa-miR-146a, hsa-miR-22, hsa-miR-382, hsa-miR-23a, hsa-miR-376c, hsa-miR-335, hsa-miR-142-5p, hsa-miR-221, hsa-miR-142-3p, hsa-miR-15 1-3p, hsa-miR-21, hsa-miR-16
Prostate Cancer	let-7d, miR-148a, miR-195, miR-25, miR-26b, miR-329, miR-376c, miR-574-3p, miR-888, miR-9, miR1204, miR-16-2*, miR-497, miR-588, miR-614, miR-765, miR92b*, miR-938, let-7f-2*, miR-300, miR-523, miR-525-5p, miR-1 182, miR-1244, miR-520d-3p, miR-379, let-7b, miR-125a-3p, miR-1296, miR-134, miR-149, miR-1 50, miR-1 87, miR-32, miR-324-3p, miR-324-5p, miR-342-3p, miR-378, miR-378*, miR-384, miR-45 1, miR-455-3p, miR-485-3p, miR-487a, miR-490-3p, miR-502-5p, miR-548a-5p, miR-550, miR-562, miR-593, miR-593*, miR-595, miR-602, miR-603, miR-654-5p, miR-877*, miR-886-5p, miR-125a-5p, miR-140-3p, miR-192, miR- 196a, miR-21 10, miR-212, miR-222, miR-224*,

PREOMICS EXHIBIT 1002
Page 1029 of 1861

	miR-30b*, miR-499-3p, miR-505*
Prostate (PCSA+ cMVs)	miR-1 82, miR-663, miR-155, mirR-125a-5p, miR-548a-5p, miR-628-5p, miR-517*, miR-450a, miR-920, hsa-miR-619, miR-1913, miR-224*, miR-502-5p, miR-888, miR-376a, miR-542-5p, miR-30b*, miR-1 179
Prostate Cancer	miR-1 83-96-1 82 cluster (miRs-1 83, 96 and 182), metal ion transporter such as hZIPl, SLC39A1, SLC39A2, SLC39A3, SLC39A4, SLC39A5, SLC39A6, SLC39A7, SLC39A8, SLC39A9, SLC39A10, SLC39A1 1, SLC39A12, SLC39A13, SLC39A14
Prostate Cancer	RAD23B, FBP1, TNFRSF1A, CCNG2, NOTCH3, ETV1, BID, SIM2, LETMD1, ANXA1, miR-5 19d, miR-647
Prostate Cancer	RAD23B, FBP1, TNFRSF1A, NOTCH3, ETV1, BID, SIM2, ANXA1, BCL2
Prostate Cancer	ANPEP, ABL1, PSCA, EFNA1, HSPB1, INMT, TRIP13
Prostate Cancer	E2F3, c-met, pRB, EZH2, e-cad, CAXII, CAIX, HIF-1α, Jagged, PIM-1, hepsin, RECK, Clusterin, MMP9, MTSP-1, MMP24, MMP15, IGFBP-2, IGFBP-3, E2F4, caveolin, EF-1A, Kallikrein 2, Kallikrein 3, PSGR
Prostate Cancer	A2ML1, BAX, C10orf47, Clorfl62, CSDA, EIFC3, ETFB, GABARAPL2, GUK1, GZMH, HIST1H3B, HLA-A, HSP90AA1, NRGN, PRDX5, PTMA, RABAC1, RABAGAP1L, RPL22, SAP 18, SEPW1, SOX1
Prostate Cancer	NY-ESO-1, SSX-2, SSX-4, XAGE-lb, AMACR, p90 autoantigen, LEDGF
Prostate Cancer	A33, ABL2, ADAM 10, AFP, ALA, ALIX, ALPL, ApoJ/CLU, ASCA, ASPH(A-10), ASPH(DOIP), AURKB, B7H3, B7H3, B7H4, BCNP, BDNF, CA125(MUC16), CA-19-9, C-Bir, CD10, CD15 1, CD24, CD41, CD44, CD46, CD59(MEM-43), CD63, CD63, CD66eCEA, CD81, CD81, CD9, CD9, CDA, CDADC1, CRMP-2, CRP, CXCL12, CXCR3, CYFRA21-1, DDX-1, DLL4, DLL4, EGFR, Epcam, EphA2, ErbB2, ERG, EZH2, FASL, FLNA, FRT, GAL3, GATA2, GM-CSF, Gro-alpha, HAP, HER3(ErbB3), HSP70, HSPB1, hVEGFR2, iC3b, IL-1B, IL6R, IL6Unc, IL7Ralpha/CD127, IL8, INSIG-2, Integrin, KLK2, LAMN, Mammoglobin, M-CSF, MFG-E8, MIF, MISRII, MMP7, MMP9, MUCl, Mucl, MUCl 7, MUC2, Ncam, NDUFB7, NGAL, NK-2R(C-21), NT5E (CD73), p53, PBP, PCSA, PCSA, PDGFRB, PIM1, PRL, PSA, PSA, PSMA, PSMA, RAGE, RANK, ReglV, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2(C-15), SPARC, SPC, SPDEF, SPP1, STEAP, STEAP4, TFF3, TGM2, TIMP-1, TMEM21 1, Trail-R2, Trail-R4, TrKB(poly), Trop2, TsglOl, TWEAK, UNC93A, VEGFA, wnt-5a(C-16)
Prostate Vesicles	CD9, CD63, CD81, PCSA, MUC2, MFG-E8
Prostate Cancer	miR-148a, miR-329, miR-9, miR-378*, miR-25, miR-614, miR-5 18c*, miR-378, miR-765, let-7f-2*, miR-574-3p, miR-497, miR-32, miR-379, miR-520g, miR-542-5p, miR-342-3p, miR-1206, miR-663, miR-222
Prostate Cancer	hsa-miR-877*, hsa-miR-593, hsa-miR-595, hsa-miR-300, hsa-miR-324-5p, hsa-miR-548a-5p, hsa-miR-329, hsa-miR-550, hsa-miR-886-5p, hsa-miR-603, hsa-miR-490-3p, hsa-miR-938, hsa-miR-149, hsa-miR-150, hsa-miR-1296, hsa-miR-384, hsa-miR-487a, hsa-miRPlus-C1089, hsa-miR-485-3p, hsa-miR-525-5p
Prostate Cancer	hsa-miR-451, hsa-miR-223, hsa-miR-593*, hsa-miR-1974, hsa-miR-486-5p, hsa-miR-19b, hsa-miR-320b, hsa-miR-92a, hsa-miR-21, hsa-miR-675*, hsa-miR-16, hsa-miR-876-5p, hsa-miR-144, hsa-miR-126, hsa-miR-137, hsa-miR-1913, hsa-miR-29b-1 *, hsa-miR-15a, hsa-miR-93, hsa-miR-1266
Inflammatory Disease	miR-588, miR-1258, miR-16-2*, miR-938, miR-526b, miR-92b*, let-7d, miR-378*, miR-124, miR-376c, miR-26b, miR-1204, miR-574-3p, miR-195, miR-499-3p, miR-21 10, miR-888
Prostate Cancer	A33, ADAM 10, AMACR, ASPH (A-10), AURKB, B7H3, CA125, CA-19-9, C-Bir, CD24, CD3, CD41, CD63, CD66e CEA, CD81, CD9, CDADC1, CSA, CXCL12, DCRN, EGFR, EphA2, ERG, FLNA, FRT, GAL3, GM-CSF, Gro-alpha, HER 3 (ErbB3), hVEGFR2, IL6 Unc, Integrin, Mammaglobin, MFG-E8, MMP9, MUCl, MUCl 7, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, PBP, PCSA, PIM1, PRL, PSA, PSIP1/LEDGF, PSMA, RANK, S100-A4, seprase/FAP, SIM2 (C-15), SPDEF, SSX2, STEAP, TGM2, TIMP-1, Trail-R4, Tsg 101, TWEAK, UNC93A, VCAN, XAGE-1
Prostate Cancer	A33, ADAM 10, ALIX, AMACR, ASCA, ASPH (A-10), AURKB, B7H3, BCNP, CA125, CA-19-9, C-Bir (Flagellin), CD24, CD3, CD41, CD63, CD66e CEA,

PREOMICS EXHIBIT 1002
Page 1030 of 1861

	CD81, CD9, CDADC1, CRP, CSA, CXCL12, CYFRA21-1, DCRN, EGFR, EpCAM, EphA2, ERG, FLNA, GAL3, GATA2, GM-CSF, Gro alpha, HER3 (ErbB3), HSP70, hVEGFR2, iC3b, IL-1B, IL6 Unc, IL8, Integrin, KLK2, Mammaglobin, MFG-E8, MMP7, MMP9, MS4A1, MUC1, MUC17, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, p53, PBP, PCSA, PIM1, PRL, PSA, PSMA, RANK, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2 (C-15), SPC, SPDEF, SSX2, SSX4, STEAP, TGM2, TIMP-1, TRAIL R2, Trail-R4, Tsg 101, TWEAK, VCAN, VEGF A, XAGE
Prostate Vesicles	EpCam, CD81, PCSA, MUC2, MFG-E8
Prostate Vesicles	CD9, CD63, CD81, MMP7, EpCAM
Prostate Cancer	let-7d, miR-148a, miR-195, miR-25, miR-26b, miR-329, miR-376c, miR-574-3p, miR-888, miR-9, miR1204, miR-16-2*, miR-497, miR-588, miR-614, miR-765, miR92b*, miR-938, let-7f-2*, miR-300, miR-523, miR-525-5p, miR-1182, miR-1244, miR-520d-3p, miR-379, let-7b, miR-125a-3p, miR-1296, miR-134, miR-149, miR-150, miR-187, miR-32, miR-324-3p, miR-324-5p, miR-342-3p, miR-378, miR-378*, miR-384, miR-451, miR-455-3p, miR-485-3p, miR-487a, miR-490-3p, miR-502-5p, miR-548a-5p, miR-550, miR-562, miR-593, miR-593*, miR-595, miR-602, miR-603, miR-654-5p, miR-877*, miR-886-5p, miR-125a-5p, miR-140-3p, miR-192, miR-196a, miR-2110, miR-212, miR-222, miR-224*, miR-30b*, miR-499-3p, miR-505*
Prostate Cancer	STAT3, EZH2, p53, MACC1, SPDEF, RUNX2, YB-1, AURKA, AURKB
Ribonucleoprotein complexes & vesicles	GW182, Ago2, miR-let-7a, miR-16, miR-22, miR-148a, miR-451, miR-92a, CD9, CD63, CD81
Prostate Cancer vesicles	PCSA, Muc2, Adam10
Prostate Cancer vesicles	Alkaline Phosphatase (AP), CD63, MyoD1, Neuron Specific Enolase, MAP1B, CNPase, Prohibitin, CD45RO, Heat Shock Protein 27, Collagen II, Laminin B1/b1, Gai1, CDw75, bcl-XL, Laminin-s, Ferritin, CD21, ADP-ribosylation Factor (ARF-6)
Prostate Cancer vesicles	CD56/NCAM-1, Heat Shock Protein 27/hsp27, CD45RO, MAP1B, MyoD1, CD45/T200/LCA, CD3zeta, Laminin-s, bcl-XL, Rad18, Gai1, Thymidylate Synthase, Alkaline Phosphatase (AP), CD63, MMP-16 / MT3-MMP, Cyclin C, Neuron Specific Enolase, SIRP a1, Laminin B1/b1, Amyloid Beta (APP), SODD (Silencer of Death Domain), CDC37, Gab-1, E2F-2, CD6, Mast Cell Chymase, Gamma Glutamylcysteine Synthetase (GCS)
Prostate Cancer vesicles	EpCAM, MMP7, PCSA, BCNP, ADAM10, KLK2, SPDEF, CD81, MFGE8, IL-8
Prostate Cancer vesicles	EpCAM, KLK2, PBP, SPDEF, SSX2, SSX4
Prostate Cancer vesicles	ADAM-10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, SSX4
Androgen Receptor (AR) pathway members in cMVs	GTF2F1, CTNNB1, PTEN, APPL1, GAPDH, CDC37, PNRC1, AES, UXT, RAN, PA2G4, JUN, BAG1, UBE2I, HDAC1, COX5B, NCOR2, STUB1, HIPK3, PXN, NCOA4
EGFR1 pathway members in cMVs	RALBP1, SH3BGRL, RBBP7, REPS1, SNRPD2, CEBPB, APPL1, MAP3K3, EEF1A1, GRB2, RAC1, SNCA, MAP2K3, CEBPA, CDC42, SH3KBP1, CBL, PTPN6, YWHAB, FOXO1, JAK1, KRT8, RALGDS, SMAD2, VAV1, NDUFA13, PRKCB1, MYC, JUN, RFXANK, HDAC1, HIST3H3, PEBP1, PXN, TNIP1, PKN2
TNF-alpha pathway members in cMVs	BCL3, SMARCE1, RPS11, CDC37, RPL6, RPL8, PAPOLA, PSMC1, CASP3, AKT2, MAP3K7IP2, POLR2L, TRADD, SMARCA4, HIST3H3, GNB2L1, PSMD1, PEBP1, HSPB1, TNIP1, RPS13, ZFAND5, YWHAQ, COMMD1, COPS3, POLR1D, SMARCC2, MAP3K3, BIRC3, UBE2D2, HDAC2, CASP8, MCM7, PSMD7, YWHAG, NFKBIA, CAST, YWHAB, G3BP2, PSMD13, FBL, RELB, YWHAZ, SKP1, UBE2D3, PDCD2, HSP90AA1, HDAC1, KPNA2, RPL30, GTF2I, PFDN2
Colorectal cancer	CD9, EGFR, NGAL, CD81, STEAP, CD24, A33, CD66E, EPHA2, Ferritin, GPR30, GPR110, MMP9, OPN, p53, TMEM211, TROP2, TGM2, TIMP, EGFR,

PREOMICS EXHIBIT 1002
Page 1031 of 1861

	DR3, UNC93A, MUC17, EpCAM, MUC1, MUC2, TSG101, CD63, B7H3
Colorectal cancer	DR3, STEAP, epha2, TMEM21 1, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, TETS
Colorectal cancer	A33, AFP, ALIX, ALX4, ANCA, APC, ASCA, AURKA, AURKB, B7H3, BANK1, BCNP, BDNF, CA-19-9, CCSA-2, CCSA-3&4, CD10, CD24, CD44, CD63, CD66 CEA, CD66e CEA, CD81, CD9, CDA, C-Erb2, CRMP-2, CRP, CRTN, CXCL12, CYFRA21-1, DcR3, DLL4, DR3, EGFR, Epcam, EphA2, FASL, FRT, GAL3, GDF15, GPCR (GPR1 10), GPR30, GRO-1, HBD 1, HBD2, HNP1-3, IL-1B, IL8, IMP3, LICAM, LAMN, MACC-1, MGC20553, MCP-1, M-CSF, MICl, MIF, MMP7, MMP9, MS4A1, MUC1, MUC17, MUC2, Ncam, NGAL, NNMT, OPN, p53, PCSA, PDGFRB, PRL, PSMA, PSME3, Reg IV, SCRNl, Sept-9, SPARC, SPON2, SPR, SRVN, TFF3, TGM2, TIMP-1, TMEM21 1, TNF-alpha, TPA, TPS, Trail-R2, Trail-R4, TrKB, TROP2, Tsg 101, TWEAK, UNC93A, VEGFA
Colorectal cancer	miR 92, miR 21, miR 9, miR 491
Colorectal cancer	miR-127-3p, miR-92a, miR-486-3p, miR-378
Colorectal cancer	TMEM21 1, MUC1, CD24 and/or GPR1 10 (GPCR_110)
Colorectal cancer	hsa-miR-376c, hsa-miR-215, hsa-miR-652, hsa-miR-582-5p, hsa-miR-324-5p, hsa-miR-1296, hsa-miR-28-5p, hsa-miR-190, hsa-miR-590-5p, hsa-miR-202, hsa-miR-195
Colorectal cancer vesicle markers	A26C1A, A26C1B, A2M, ACAA2, ACE, ACOT7, ACPI, ACTA1, ACTA2, ACTB, ACTBL2, ACTBL3, ACTC1, ACTG1, ACTG2, ACTN1, ACTN2, ACTN4, ACTR3, ADAM 10, ADSL, AGR2, AGR3, AGRN, AHCY, AHNAK, AKR1B10, ALB, ALDH16A1, ALDH1A1, ALDOA, ANXAl, ANXAl 1, ANXA2, ANXA2P2, ANXA4, ANXA5, ANXA6, AP2A1, AP2A2, APOA1, ARF1, ARF3, ARF4, ARF5, ARF6, ARHGDIA, ARPC3, ARPC5L, ARRDC1, ARVCF, ASCC3L1, ASNS, ATP1A1, ATP1A2, ATP 1A3, ATP1B1, ATP4A, ATP5A1, ATP5B, ATP5I, ATP5L, ATP50, ATP6AP2, B2M, BAIAP2, BAIAP2L1, BRI3BP, BSG, BUB3, Clorf58, C5orG2, CAD, CALM1, CALM2, CALM3, CANDl, CANX, CAPZAl, CBRl, CBR3, CCT2, CCT3, CCT4, CCT5, CCT6A, CCT7, CCT8, CD44, CD46, CD55, CD59, CD63, CD81, CD82, CD9, CDC42, CDH1, CDH17, CEACAM5, CFL1, CFL2, CHMP1A, CHMP2A, CHMP4B, CKB, CLDN3, CLDN4, CLDN7, CLIC1, CLIC4, CLSTN1, CLTC, CLTCL1, CLU, COL12A1, COPB1, COPB2, COROIC, COX4I1, COX5B, CRYZ, CSPG4, CSRPl, CST3, CTNNAl, CTNNBl, CTNNDl, CTTN, CYFIPl, DCD, DERA, DIP2A, DIP2B, DIP2C, DMBT1, DPEP1, DPP4, DYNC1H1, EDIL3, EEF1A1, EEF1A2, EEF1AL3, EEF 1G, EEF2, EFNB1, EGFR, EHD1, EHD4, EIF3EIP, EIF3I, EIF4A1, EIF4A2, ENOl, EN02, EN03, EPHA2, EPHA5, EPHB1, EPHB2, EPHB3, EPHB4, EPPK1, ESD, EZR, F11R, F5, F7, FAM125A, FAM125B, FAM129B, FASLG, FASN, FAT, FCGBP, FER1L3, FKBP1A, FLNA, FLNB, FLOT1, FLOT2, G6PD, GAPDH, GARS, GCN1L1, GDI2, GK, GMDS, GNA13, GNAI2, GNAI3, GNAS, GNB1, GNB2, GNB2L1, GNB3, GNB4, GNG12, GOLGA7, GPA33, GPI, GPRC5A, GSN, GSTP1, H2AFJ, HADHA, hCG_1757335, HEPH, HIST1H2AB, HIST1H2AE, HIST1H2AJ, HIST1H2AK, HIST1H4A, HIST1H4B, HIST1H4C, HIST1H4D, HIST1H4E, HIST1H4F, HIST1H4H, HIST1H4I, HIST1H4J, HIST1H4K, HIST1H4L, HIST2H2AC, HIST2H4A, HIST2H4B, HIST3H2A, HIST4H4, HLA-A, HLA-A29.1, HLA-B, HLA-C, HLA-E, HLA-H, HNRNPA2B1, HNRNPH2, HPCAL1, HRAS, HSD17B4, HSP90AA1, HSP90AA2, HSP90AA4P, HSP90AB1, HSP90AB2P, HSP90AB3P, HSP90B1, HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA4, HSPA5, HSPA6, HSPA7, HSPA8, HSPA9, HSPD1, HSPE1, HSPG2, HYOU1, IDH1, IFITM1, IFITM2, IFITM3, IGH@, IGHG1, IGHG2, IGHG3, IGHG4, IGHM, IGHV4-3 1, IGK@, IGKC, IGKV1-5, IGKV2-24, IGKV3-20, IGSF3, IGSF8, IQGAP1, IQGAP2, ITGA2, ITGA3, ITGA6, ITGAV, ITGB1, ITGB4, JUP, KIAA0174, KIAA1 199, KPNB1, KRAS, KRT1, KRT10, KRT13, KRT14, KRT15, KRT16, KRT17, KRT18, KRT19, KRT2, KRT20, KRT24, KRT25, KRT27, KRT28, KRT3, KRT4, KRT5, KRT6A, KRT6B, KRT6C, KRT7, KRT75, KRT76, KRT77, KRT79, KRT8, KRT9, LAMA5, LAMP1, LDHA, LDHB, LFNG, LGALS3, LGALS3BP, LGALS4, LIMA1, LIN7A, LIN7C, LOC100128936, LOC100130553, LOC100133382,

PREOMICS EXHIBIT 1002
Page 1032 of 1861

	LOC100133739, LOC284889, LOC388524, LOC388720, LOC442497, LOC653269, LRP4, LRPPRC, LRSAM1, LSR, LYZ, MANIAI, MAP4K4, MARCKS, MARCKSL1, METRNL, MFGE8, MICA, MIF, MINK1, MITD1, MMP7, MOBKL1A, MSN, MTCH2, MUC13, MYADM, MYH10, MYH1 1, MYH14, MYH9, MYL6, MYL6B, MYOIC, MYOID, NARS, NCALD, NCSTN, NEDD4, NEDD4L, NME1, NME2, NOTCH 1, NQOl, NRAS, P4HB, PCBP1, PCNA, PCSK9, PDCD6, PDCD6IP, PDIA3, PDXK, PEBP1, PFN1, PGK1, PHB, PHB2, PKM2, PLEC1, PLEKHB2, PLSCR3, PLXNA1, PLXNB2, PPIA, PPIB, PPP2R1A, PRDX1, PRDX2, PRDX3, PRDX5, PRDX6, PRKAR2A, PRKDC, PRSS23, PSMA2, PSMC6, PSMD1 1, PSMD3, PSME3, PTGFRN, PTPRF, PYGB, QPCT, QSOX1, RAB10, RAB1 1A, RAB1 1B, RAB13, RAB14, RAB15, RAB1A, RAB1B, RAB2A, RAB33B, RAB35, RAB43, RAB4B, RAB5A, RAB5B, RAB5C, RAB6A, RAB6B, RAB7A, RAB8A, RAB8B, RAC1, RAC3, RALA, RALB, RAN, RANP1, RAP1A, RAP IB, RAP2A, RAP2B, RAP2C, RDX, REG4, RHOA, RHOC, RHOG, ROCK2, RP1 1-63 1M21 .2, RPL10A, RPL12, RPL6, RPL8, RPLPO, RPLPO-like, RPLP1, RPLP2, RPN1, RPS13, RPS14, RPS15A, RPS16, RPS1 8, RPS20, RPS21, RPS27A, RPS3, RPS4X, RPS4Y1, RPS4Y2, RPS7, RPS8, RPSA, RPSAP15, RRAS, RRAS2, RUVBL1, RUVBL2, S100A10, S100A1 1, S100A14, S100A16, S100A6, S100P, SDC1, SDC4, SDCBP, SDCBP2, SERINC1, SERINC5, SERPINA1, SERPINF1, SETD4, SFN, SLC12A2, SLC12A7, SLC16A1, SLC1A5, SLC25A4, SLC25A5, SLC25A6, SLC29A1, SLC2A1, SLC3A2, SLC44A1, SLC7A5, SLC9A3R1, SMPDL3B, SNAP23, SND1, SOD1, SORT1, SPTAN1, SPTBN1, SSBP1, SSR4, TACSTD1, TAGLN2, TBCA, TCEB1, TCP1, TF, TFRC, THBS1, TJP2, TKT, TMED2, TNFSF 10, TNIK, TNKS1BP1, TNP03, TOLLIP, TOMM22, TPI1, TPM1, TRAP1, TSG101, TSPAN1, TSPAN14, TSPAN15, TSPAN6, TSPAN8, TSTA3, TTYH3, TUBA 1A, TUBA IB, TUBA1C, TUBA3C, TUBA3D, TUBA3E, TUBA4A, TUBA4B, TUBA8, TUBB, TUBB2A, TUBB2B, TUBB2C, TUBB3, TUBB4, TUBB4Q, TUBB6, TUFM, TXN, UBA1, UBA52, UBB, UBC, UBE2N, UBE2V2, UGDH, UQCRC2, VAMP1, VAMP3, VAMP8, VCP, VIL1, VPS25, VPS28, VPS35, VPS36, VPS37B, VPS37C, WDRl, YWHAB, YWHAE, YWHAG, YWHAH, YWHAQ, YWHAZ
Colorectal Cancer	hsa-miR-16, hsa-miR-25, hsa-miR-125b, hsa-miR-45 1, hsa-miR-200c, hsa-miR-140-3p, hsa-miR-658, hsa-miR-370, hsa-miR-1296, hsa-miR-636, hsa-miR-502-5p
Breast cancer	miR-21, miR-155, miR-206, miR-122a, miR-210, miR-21, miR-155, miR-206, miR-122a, miR-210, let-7, miR-lOb, miR-125a, miR-125b, miR-145, miR-143, miR-145, miR-lb
Breast cancer	GAS5
Breast cancer	ER, PR, HER2, MUCl, EGFR, KRAS, B-Raf, CYP2D6, hsp70, MART-1, TRP, HER2, hsp70, MART-1, TRP, HER2, ER, PR, Class III b-tubulin, VEGFA, ETV6-NTRK3, BCA-225, hsp70, MARTI, ER, VEGFA, Class III b-tubulin, HER2/neu (e.g., for Her2+ breast cancer), GPR30, ErbB4 (JM) isoform, MPR8, MISIIR, CD9, EphA2, EGFR, B7H3, PSM, PCSA, CD63, STEAP, CD81, KAMI, A33, DR3, CD66e, MFG-E8, TROP-2, Mammaglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, EpCam, neurokinin receptor- 1 (NK-1 or NK-1R), NK-2, Pai-1, CD45, CD10, HER2/ERBB2, AGTR1, NPY1R, MUCl, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUCl secreted), CA27.29 (MUCl secreted), NMDAR1, NMDAR2, MAGEA, CTAG1B, NY-ESO-1, SPB, SPC, NSE, PGP9.5, progesterone receptor (PR) or its isoform (PR(A) or PR(B)), P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, IC3b, mesothelin, SPA, AQP5, GPCR, hCEA-CAM, PTP IA-2, CABYR, TMEM21 1, ADAM28, UNC93A, MUCl 7, MUC2, ILlOR-beta, BCMA, HVEM/TNFRSF14, Trappin-2, Elafin, ST2/IL1 R4, TNFRF14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNFR
Breast cancer	CD9, MIS Rii, ER, CD63, MUCl, HER3, STAT3, VEGFA, BCA, CA125, CD24, EPCAM, ERB B4
Breast cancer	CD10, NPGP/NPFF2, HER2/ERBB2, AGTR1, NPY1R, neurokinin receptor-1 (NK-1 or NK-1R), NK-2, MUCl, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUCl secreted), CA27.29 (MUCl secreted), NMDAR1, NMDAR2, MAGEA,

PREOMICS EXHIBIT 1002
Page 1033 of 1861

	CTAG1B, NY-ESO-1
Breast cancer	SPB, SPC, NSE, PGP9.5, CD9, P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, EGFR, B7H3, IC3b, MUC1, mesothelin, SPA, PCSA, CD63, STEAP, AQP5, CD81, DR3, PSM, GPCR, EphA2, hCEA-CAM, PTP IA-2, CABYR, TMEM21 1, ADAM28, UNC93A, A33, CD24, CD10, NGAL, EpCam, MUC17, TROP-2, MUC2, ILlOR-beta, BCMA, HVEM/TNFRSF14, Trappin-2 Elafm, ST2/IL1 R4, TNFRF14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNFR
Breast cancer	BRCA, MUC-1, MUC 16, CD24, ErbB4, ErbB2 (HER2), ErbB3, HSP70, Mammaglobin, PR, PR(B), VEGFA
Breast cancer	CD9, HSP70, Gal3, MIS, EGFR, ER, ICB3, CD63, B7H4, MUC1, DLL4, CD81, ERB3, VEGF, BCA225, BRCA, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD3 1, cMET, MUC2, ERBB4
Breast cancer	CD9, EphA2, EGFR, B7H3, PSMA, PCSA, CD63, STEAP, CD81, STEAP1, ICAM1 (CD54), PSMA, A33, DR3, CD66e, MFG-8e, TMEM21 1, TROP-2, EGFR, Mammoglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, NK-2, EpCam, NK-1R, PSMA, 5T4, PAI-1, CD45
Breast cancer	PGP9.5, CD9, HSP70, gal3-b2cl0, EGFR, iC3b, PSMA, PCSA, CD63, MUC1, DLL4, CD81, B7-H3, HER 3 (ErbB3), MART-1, PSA, VEGF A, TIMP-1, GPCR GPR1 10, EphA2, MMP9, mmp7, TMEM21 1, UNC93a, BRCA, CA125 (MUC 16), Mammaglobin, CD174 (Lewis y), CD66e CEA, CD24 c.sn3, C-erbB2, CD 10, NGAL, epcam, CEA (carcinoembryonic Antigen), GPR30, CYFRA21-1, OPN, MUC 17, hVEGFR2, MUC2, NCAM, ASPH, ErbB4, SPB, SPC, CD9, MS4A1, EphA2, MIS RII, HER2 (ErbB2), ER, PR (B), MRP8, CD63, B7H4, TGM2, CD81, DR3, STAT 3, MACC-1, TrKB, IL 6 Unc, OPG - 13, IL6R, EZH2, SCRNl, TWEAK, SERPINB3, CDAC1, BCA-225, DR3, A33, NPGP/NPFF2, TIMP1, BDNF, FRT, Ferritin heavy chain, seprase, p53, LDH, HSP, ost, p53, CXCL12, HAP, CRP, Gro-alpha, Tsg 101, GDF15
Breast cancer	CD9, HSP70, Gal3, MIS (RII), EGFR, ER, ICB3, CD63, B7H4, MUC1, CD81, ERB3, MARTI, STAT3, VEGF, BCA225, BRCA, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD3 1, cMET, MUC2, ERB4, TMEM21 1
Breast Cancer	5T4 (trophoblast), ADAM 10, AGER/RAGE, APC, APP (β-amyloid), ASPH (A-10), B7H3 (CD276), BACE1, BAD, BRCA1, BDNF, BIRC2, C1GALT1, CA125 (MUC 16), Calmodulin 1, CCL2 (MCP-1), CD9, CD 10, CD 127 (IL7R), CD 174, CD24, CD44, CD63, CD81, CEA, CRMP-2, CXCR3, CXCR4, CXCR6, CYFRA 21, derlin 1, DLL4, DPP6, E-CAD, EpCaM, EphA2 (H-77), ER(1) ESRl a, ER(2) ESR2 β, Erb B4, Erbb2, erb3 (Erb-B3), PA2G4, FRT (FLT1), Gal3, GPR30 (G-coupled ER1), HAP1, HER3, HSP-27, HSP70, IC3b, IL8, insig, junction plakoglobin, Keratin 15, KRAS, Mammaglobin, MARTI, MCT2, MFGE8, MMP9, MRP 8, Mucl, MUC 17, MUC2, NCAM, NG2 (CSPG4), Ngal, NHE-3, NT5E (CD73), ODC1, OPG, OPN, p53, PARK7, PCSA, PGP9.5 (PARK5), PR(B), PSA, PSMA, RAGE, STXBP4, Survivin, TFF3 (secreted), TIMP1, TIMP2, TMEM21 1, TRAF4 (scaffolding), TRAIL-R2 (death Receptor 5), TrkB, Tsg 101, UNC93a, VEGF A, VEGFR2, YB-1, VEGFR1, GCDPF-15 (PIP), BigH3 (TGFbl -induced protein), 5HT2B (serotonin receptor 2B), BRCA2, BACE 1, CDHl-cadherin
Breast Cancer	AK5.2, ATP6V1B1, CRABP1
Breast Cancer	DST.3, GATA3, KRT81
Breast Cancer	AK5.2, ATP6V1B1, CRABP1, DST.3, ELF5, GATA3, KRT81, LALBA, OXTR, RASL10A, SERHL, TFAP2A. 1, TFAP2A.3, TFAP2C, VTCN1
Breast Cancer	TRAP; Renal Cell Carcinoma; Filamin; 14.3.3, Pan; Prohibitin; c-fos; Ang-2; GSTmu; Ang-1 ; FHIT; Rad51 ; Inhibin alpha; Cadherin-P; 14.3.3 gamma; p18INK4c; P504S; XRCC2; Caspase 5; CREB-Binding Protein; Estrogen Receptor; IL17; Claudin 2; Keratin 8; GAPDH; CD1 ; Keratin, LMW; Gamma Glutamylcysteine Synthetase(GCS)/Glutamate-cysteine Ligase; a-B-Crystallin; Pax-5; MMP-19; APC; IL-3; Keratin 8 (phospho-specific Ser73); TGF-beta 2; ITK; Oct-2/; DJ-1 ; B7-H2; Plasma Cell Marker; Radl 8; Estriol; Chkl ; Prolactin Receptor; Laminin Receptor; Histone H1; CD45RO; GnRH Receptor; IP10/CRG2; Actin, Muscle Specific; S100; Dystrophin; Tubulin-a; CD3zeta; CDC37; GABA a Receptor 1; MMP-7 (Matrilysin); Heregulin; Caspase 3;

PREOMICS EXHIBIT 1002
Page 1034 of 1861

	CD56/NCAM-1; Gastrin 1; SREBP-1 (Sterol Regulatory Element Binding Protein- 1); MLHl; PGP9.5; Factor VIII Related Antigen; ADP-ribosylation Factor (ARF-6); MHC II (HLA-DR) la; Survivin; CD23; G-CSF; CD2; Cabetinin; Neuron Specific Enolase; CD 165; Calponin; CD95 / Fas; Urocortin; Heat Shock Protein 27/hsp27; Topo II beta; Insulin Receptor; Keratin 5/8; sm; Actin, skeletal muscle; CA19-9; GluRl; GRIP1; CD79amb-l; TdT; HRP; CD94; CCK-8; Thymidine Phosphorylase; CD57; Alkaline Phosphatase (AP); CD59 / MACIF / MIRL / Protectin; GLUT-1; alpha- 1-antitrypsin; Presenillin; Mucin 3 (MUC3); pS2; 14-3-3 beta; MMP-13 (Collagenase-3); Fli-1; mGluR5; Mast Cell Chymase; Laminin Bl/bl; Neurofilament (160kDa); CNPase; Amylin Peptide; Gail; CD6; alpha- 1-antichymotrypsin; E2F-2; MyoDl
Ductal carcinoma in situ (DCIS)	Laminin Bl/bl; E2F-2; TdT; Apolipoprotein D; Granulocyte; Alkaline Phosphatase (AP); Heat Shock Protein 27/hsp27; CD95 / Fas; pS2; Estriol; GLUT-1; Fibronectin; CD6; CCK-8; sm; Factor VIII Related Antigen; CD57; Plasminogen; CD71 / Transferrin Receptor; Keratin 5/8; Thymidine Phosphorylase; CD45/T200/LCA; Epithelial Specific Antigen; Macrophage; CD10; MyoDl; Gail; bcl-XL; hPL; Caspase 3; Actin, skeletal muscle; IP10/CRG2; GnRH Receptor; p35nck5a; ADP-ribosylation Factor (ARF-6); Cdk4 ; alpha- 1-antitrypsin; IL17; Neuron Specific Enolase; CD56/NCAM-1; Prolactin Receptor; Cdk7; CD79amb-l; Collagen IV; CD94; Myeloid Specific Marker; Keratin 10; Pax-5; IgM (m-Heavy Chain); CD45RO; CA19-9; Mucin 2; Glucagon; Mast Cell Chymase; MLHl; CD1; CNPase; Parkin; MHC II (HLA-DR) la; B7-H2; Chkl; Lambda Light Chain; MHC II (HLA-DP and DR); Myogenin; MMP-7 (Matrilysin); Topo II beta; CD53; Keratin 19; Radl8; Ret Oncoprotein; MHC II (HLA-DP); E3-binding protein (ARM1); Progesterone Receptor; Keratin 8; IgG; IgA; Tubulin; Insulin Receptor Substrate-1; Keratin 15; DR3; IL-3; Keratin 10/13; Cyclin D3; MHC I (HLA25 and HLA-Aw32); Calmodulin; Neurofilament (160kDa)
Ductal carcinoma in situ (DCIS) v. other Breast cancer	Macrophage; Fibronectin; Granulocyte; Keratin 19; Cyclin D3; CD45/T200/LCA; EGFR; Thrombospondin; CD81/TAPA-1; Ruv C; Plasminogen; Collagen IV; Laminin Bl/bl; CD10; TdT; Filamin; bcl-XL; 14.3.3 gamma; 14.3.3, Pan; pl70; Apolipoprotein D; CD71 / Transferrin Receptor; FHIT
Lung cancer	Pgrmcl (progesterone receptor membrane component l)/sigma-2 receptor, STEAP, EZH2
Lung cancer	Prohibitin, CD23, Amylin Peptide, HRP, Rad51, Pax-5, Oct-3/, GLUT-1, PSCA, Thrombospondin, FHIT, a-B-Crystallin, LewisA, Vacular Endothelial Growth Factor(VEGF), Hepatocyte Factor Homologue-4, Flt-4, GluR6/7, Prostate Apoptosis Response Protein-4, GluRl, Fli-1, Urocortin, S100A4, 14-3-3 beta, P504S, HDAC1, PGP9.5, DJ-1, COX2, MMP-19, Actin, skeletal muscle, Claudin 3, Cadherin-P, Collagen IX, p27Kipl, Cathepsin D, CD30 (Reed- Sternberg Cell Marker), Ubiquitin, FSH-b, TrxR2, CCK-8, Cyclin C, CD138, TGF-beta 2, Adrenocorticotrophic Hormone, PPAR-gamma, Bcl-6, GLUT-3, IGF-I, mRANKL, Fas-ligand, Filamin, Calretinin, O ct-1, Parathyroid Hormone, Claudin 5, Claudin 4, Raf-1 (Phospho-specific), CDC14A Phosphatase, Mitochondria, APC, Gastrin 1, Ku (p80), Gail, XPA, Maltose Binding Protein, Melanoma (gplOO), Phosphotyrosine, Amyloid A, CXCR4 / Fusin, Hepatic Nuclear Factor-SB, Caspase 1, HPV 16-E7, Axonal Growth Cones, Lck, Ornithine Decarboxylase, Gamma Glutamylcysteine Synthetase(GCS)/Glutamate-cysteine Ligase, ERCC1, Calmodulin, Caspase 7 (Mch 3), CD137 (4-1BB), Nitric Oxide Synthase, brain (bNOS), E2F-2, IL-10R, L-Plastin, CD 18, Vimentin, CD50/ICAM-3, Superoxide Dismutase, Adenovirus Type 5 E1A, PHAS-I, Progesterone Receptor (phospho-specific) - Serine 294, MHC II (HLA-DQ), XPG, ER Ca+2 ATPase2, Laminin-s, E3-binding protein (ARM1), CD45RO, CD1, Cdk2, MMP-10 (Stromilysin-2), sm, Surfactant Protein B (Pro), Apolipoprotein D, CD46, Keratin 8 (phospho-specific Ser73), PCNA, PLAP, CD20, Syk, LH, Keratin 19, ADP-ribosylation Factor (ARF-6), Int-2 Oncoprotein, Luciferase, AIF (Apoptosis Inducing Factor), Grb2, bcl-X, CD 16, Paxillin, MHC II (HLA-DP and DR), B-Cell, p21WAFl, MHC II (HLA-DR), Tyrosinase, E2F-1, Pdsl, Calponin, Notch, CD26/DPP IV, SV40 Large T Antigen, Ku (p70/p80), Perforin, XPF, SIM Ag (SIMA-4D3), Cdkl/p34cdc2, Neuron Specific Enolase, b-2-Microglobulin,

PREOMICS EXHIBIT 1002
Page 1035 of 1861

	DNA Polymerase Beta, Thyroid Hormone Receptor, Human, Alkaline Phosphatase (AP), Plasma Cell Marker, Heat Shock Protein 70/hsp70, TRP75 / gp75, SRF (Serum Response Factor), Laminin Bl/bl, Mast Cell Chymase, Caldesmon, CEA / CD66e, CD24, Retinoid X Receptor (hRXR), CD45/T200/LCA, Rabies Virus, Cytochrome c, DR3, bcl-XL, Fascin, CD71 / Transferrin Receptor
Lung Cancer	miR-497
Lung Cancer	Pgrmcl
Ovarian Cancer	CA-125, CA 19-9, c-reactive protein, CD95(also called Fas, Fas antigen, Fas receptor, FasR, TNFRSF6, APT1 or APO-1), FAP-1, miR-200 microRNAs, EGFR, EGFRvIII, apolipoprotein AI, apolipoprotein CIII, myoglobin, tenascin C, MSH6, claudin-3, claudin-4, caveolin-1, coagulation factor III, CD9, CD36, CD37, CD53, CD63, CD81, CD136, CD147, Hsp70, Hsp90, Rabl3, Desmocollin-1, EMP-2, CK7, CK20, GCDF15, CD82, Rab-5b, Annexin V, MFG-E8, HLA-DR. MiR-200 microRNAs (miR-200a, miR-200b, miR-200c), miR-141, miR-429, JNK, Jun
Prostate Cancer v normal	AQP2, BMP5, C16orf86, CXCL13, DST, ERCC1, GNAOl, KLHL5, MAP4K1, NELL2, PENK, PGF, POU3F1, PRSS21, SCML1, SEMG1, SMARCD3, SNAI2, TAF1C, TNNT3
Prostate Cancer v Breast Cancer	ADRB2, ARG2, C22orG2, CYorfl4, EIF1AY, FEV, KLK2, KLK4, LRRC26, MAOA, NLGN4Y, PNPLA7, PVRL3, SIM2, SLC30A4, SLC45A3, STX19, TRIM36, TRPM8
Prostate Cancer v Colorectal Cancer	ADRB2, BAIAP2L2, C19orG3, CDXl, CEACAM6, EEF1A2, ERN2, FAMllOB, FOXA2, KLK2, KLK4, LOC389816, LRRC26, MIPOL1, SLC45A3, SPDEF, TRIM31, TRIM36, ZNF613
Prostate Cancer v Lung Cancer	ASTN2, CAB39L, CRIPl, FAMllOB, FEV, GSTPl, KLK2, KLK4, LOC389816, LRRC26, MUC1, PNPLA7, SIM2, SLC45A3, SPDEF, TRIM36, TRPV6, ZNF613
Prostate Cancer	miRs-26a+b, miR-15, miR-16, miR-195, miR-497, miR-424, miR-206, miR-342-5p, miR-186, miR-1271, miR-600, miR-216b, miR-519 family, miR-203
Integrins	ITGA1 (CD49a, VLA1), ITGA2 (CD49b, VLA2), ITGA3 (CD49c, VLA3), ITGA4 (CD49d, VLA4), ITGA5 (CD49e, VLA5), ITGA6 (CD49f, VLA6), ITGA7 (FLJ25220), ITGA8, ITGA9 (RLC), ITGA10, ITGA11 (HsT18964), ITGAD (CD11D, FLJ39841), ITGAE (CD103, HUMINAE), ITGAL (CDlla, LFA1A), ITGAM (CD1 lb, MAC-1), ITGAV (CD51, VNRA, MSK8), ITGAW, ITGAX (CDllc), ITGB1 (CD29, FNRB, MSK12, MDF20), ITGB2 (CD18, LFA-1, MAC-1, MFI7), ITGB3 (CD61, GP3A, GPIIIa), ITGB4 (CD 104), ITGB5 (FLJ26658), ITGB6, ITGB7, ITGB8
Glycoprotein	GpIa-IIa, GpIIb-IIIa, GpIIIb, Gplb, GpIX
Transcription factors	STAT3, EZH2, p53, MACC1, SPDEF, RUNX2, YB-1
Kinases	AURKA, AURKB
Disease Markers	6Ckine, Adiponectin, Adrenocorticotropic Hormone, Agouti-Related Protein, Aldose Reductase, Alpha- 1-Antichymotrypsin, Alpha- 1-Antitrypsin, Alpha- 1-Microglobulin, Alpha-2-Macroglobulin, Alpha-Fetoprotein, Amphiregulin, Angiogenin, Angiopoietin-2, Angiotensin-Converting Enzyme, Angiotensinogen, Annexin Al, Apolipoprotein A-I, Apolipoprotein A-II, Apolipoprotein A-IV, Apolipoprotein B, Apolipoprotein C-I, Apolipoprotein C-III, Apolipoprotein D, Apolipoprotein E, Apolipoprotein H, Apolipoprotein(a), AXL Receptor Tyrosine Kinase, B cell-activating Factor, B Lymphocyte Chemoattractant, Bcl-2-like protein 2, Beta-2-Microglobulin, Betacellulin, Bone Morphogenetic Protein 6, Brain-Derived Neurotrophic Factor, Calbindin, Calcitonin, Cancer Antigen 125, Cancer Antigen 15-3, Cancer Antigen 19-9, Cancer Antigen 72-4, Carcinoembryonic Antigen, Cathepsin D, CD 40 antigen, CD40 Ligand, CD5 Antigen-like, Cellular Fibronectin, Chemokine CC-4, Chromogranin-A, Ciliary Neurotrophic Factor, Clusterin, Collagen IV, Complement C3, Complement Factor H, Connective Tissue Growth Factor, Cortisol, C-Peptide, C-Reactive Protein, Creatine Kinase-MB, Cystatin-C, Endoglin, Endostatin, Endothelin-1, EN-RAGE, Eotaxin-1, Eotaxin-2, Eotaxin-3, Epidermal Growth Factor, Epiregulin, Epithelial cell adhesion molecule, Epithelial-Derived Neutrophil-Activating Protein 78, Erythropoietin, E-Selectin, Ezrin, Factor VII, Fas Ligand,

PREOMICS EXHIBIT 1002
Page 1036 of 1861

	FASLG Receptor, Fatty Acid-Binding Protein (adipocyte), Fatty Acid-Binding Protein (heart), Fatty Acid-Binding Protein (liver), Ferritin, Fetuin-A, Fibrinogen, Fibroblast Growth Factor 4, Fibroblast Growth Factor basic, Fibulin-lC, Follicle-Stimulating Hormone, Galectin-3, Gelsolin, Glucagon, Glucagon-like Peptide 1, Glucose-6-phosphate Isomerase, Glutamate-Cysteine Ligase Regulatory subunit, Glutathione S-Transferase alpha, Glutathione S-Transferase Mu 1, Granulocyte Colony-Stimulating Factor, Granulocyte-Macrophage Colony-Stimulating Factor, Growth Hormone, Growth-Regulated alpha protein, Haptoglobin, HE4, Heat Shock Protein 60, Heparin-Binding EGF-Like Growth Factor, Hepatocyte Growth Factor, Hepatocyte Growth Factor Receptor, Hepsin, Human Chorionic Gonadotropin beta, Human Epidermal Growth Factor Receptor 2, Immunoglobulin A, Immunoglobulin E, Immunoglobulin M, Insulin, Insulin-like Growth Factor I, Insulin-like Growth Factor-Binding Protein 1, Insulin-like Growth Factor-Binding Protein 2, Insulin-like Growth Factor-Binding Protein 3, Insulin-like Growth Factor Binding Protein 4, Insulin-like Growth Factor Binding Protein 5, Insulin-like Growth Factor Binding Protein 6, Intercellular Adhesion Molecule 1, Interferon gamma, Interferon gamma Induced Protein 10, Interferon-inducible T-cell alpha chemoattractant, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-2, Interleukin-2 Receptor alpha, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-6 Receptor, Interleukin-6 Receptor subunit beta, Interleukin-7, Interleukin-8, Interleukin-10, Interleukin-11, Interleukin-12 Subunit p40, Interleukin-12 Subunit p70, Interleukin-13, Interleukin-15, Interleukin-16, Interleukin-25, Kallikrein 5, Kallikrein-7, Kidney Injury Molecule-1, Lactoylglutathione lyase, Latency-Associated Peptide of Transforming Growth Factor beta 1, Lectin-Like Oxidized LDL Receptor 1, Leptin, Luteinizing Hormone, Lymphotactin, Macrophage Colony-Stimulating Factor 1, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Macrophage Inflammatory Protein-3 alpha, Macrophage inflammatory protein 3 beta, Macrophage Migration Inhibitory Factor, Macrophage-Derived Chemokine, Macrophage-Stimulating Protein, Malondialdehyde-Modified Low-Density Lipoprotein, Maspin, Matrix Metalloproteinase-1, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-7, Matrix Metalloproteinase-9, Matrix Metalloproteinase -9, Matrix Metalloproteinase-10, Mesothelin, MHC class I chain-related protein A, Monocyte Chemotactic Protein 1, Monocyte Chemotactic Protein 2, Monocyte Chemotactic Protein 3, Monocyte Chemotactic Protein 4, Monokine Induced by Gamma Interferon, Myeloid Progenitor Inhibitory Factor 1, Myeloperoxidase, Myoglobin, Nerve Growth Factor beta, Neuronal Cell Adhesion Molecule, Neuron-Specific Enolase, Neuropilin-1, Neutrophil Gelatinase-Associated Lipocalin, NT-proBNP, Nucleoside diphosphate kinase B, Osteopontin, Osteoprotegerin, Pancreatic Polypeptide, Pepsinogen I, Peptide YY, Peroxiredoxin-4, Phosphoserine Aminotransferase, Placenta Growth Factor, Plasminogen Activator Inhibitor 1, Platelet-Derived Growth Factor BB, Pregnancy-Associated Plasma Protein A, Progesterone, Proinsulin (inc. Total or Intact), Prolactin, Prostasin, Prostate-Specific Antigen (inc. Free PSA), Prostatic Acid Phosphatase, Protein S100-A4, Protein S100-A6, Pulmonary and Activation-Regulated Chemokine, Receptor for advanced glycosylation end products, Receptor tyrosine-protein kinase erbB-3, Resistin, SI00 calcium-binding protein B, Secretin, Serotransferrin, Serum Amyloid P-Component, Serum Glutamic Oxaloacetic Transaminase, Sex Hormone-Binding Globulin, Sortilin, Squamous Cell Carcinoma Antigen-1, Stem Cell Factor, Stromal cell-derived Factor-1, Superoxide Dismutase 1 (soluble), T Lymphocyte-Secreted Protein 1-309, Tamm-Horsfall Urinary Glycoprotein, T-Cell-Specific Protein RANTES, Tenascin-C, Testosterone, Tetranectin, Thrombomodulin, Thrombopoietin, Thrombospondin-1, Thyroglobulin, Thyroid-Stimulating Hormone, Thyroxine-Binding Globulin, Tissue Factor, Tissue Inhibitor of Metalloproteinases 1, Tissue type Plasminogen activator, TNF-Related Apoptosis-Inducing Ligand Receptor 3, Transforming Growth Factor alpha, Transforming Growth Factor beta-3, Transthyretin, Trefoil Factor 3, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Tumor Necrosis Factor Receptor I, Tumor necrosis Factor Receptor 2, Tyrosine kinase

PREOMICS EXHIBIT 1002
Page 1037 of 1861

	with Ig and EGF homology domains 2, Urokinase-type Plasminogen Activator, Urokinase-type plasminogen activator Receptor, Vascular Cell Adhesion Molecule-1, Vascular Endothelial Growth Factor, Vascular endothelial growth Factor B, Vascular Endothelial Growth Factor C, Vascular endothelial growth Factor D, Vascular Endothelial Growth Factor Receptor 1, Vascular Endothelial Growth Factor Receptor 2, Vascular endothelial growth Factor Receptor 3, Vitamin K-Dependent Protein S, Vitronectin, von Willebrand Factor, YKL-40
Disease Markers	Adiponectin, Adrenocorticotropic Hormone, Agouti-Related Protein, Alpha-1-Antichymotrypsin, Alpha-1-Antitrypsin, Alpha-1-Microglobulin, Alpha-2-Macroglobulin, Alpha-Fetoprotein, Amphiregulin, Angiopoietin-2, Angiotensin-Converting Enzyme, Angiotensinogen, Apolipoprotein A-I, Apolipoprotein A-II, Apolipoprotein A-IV, Apolipoprotein B, Apolipoprotein C-I, Apolipoprotein C-III, Apolipoprotein D, Apolipoprotein E, Apolipoprotein H, Apolipoprotein(a), AXL Receptor Tyrosine Kinase, B Lymphocyte Chemoattractant, Beta-2-Microglobulin, Betacellulin, Bone Morphogenetic Protein 6, Brain-Derived Neurotrophic Factor, Calbindin, Calcitonin, Cancer Antigen 125, Cancer Antigen 19-9, Carcinoembryonic Antigen, CD 40 antigen, CD40 Ligand, CD5 Antigen-like, Chemokine CC-4, Chromogranin-A, Ciliary Neurotrophic Factor, Clusterin, Complement C3, Complement Factor H, Connective Tissue Growth Factor, Cortisol, C-Peptide, C-Reactive Protein, Creatine Kinase-MB, Cystatin-C, Endothelin-1, EN-RAGE, Eotaxin-1, Eotaxin-3, Epidermal Growth Factor, Epiregulin, Epithelial-Derived Neutrophil-Activating Protein 78, Erythropoietin, E-Selectin, Factor VII, Fas Ligand, FASLG Receptor, Fatty Acid-Binding Protein (heart), Ferritin, Fetuin-A, Fibrinogen, Fibroblast Growth Factor 4, Fibroblast Growth Factor basic, Follicle-Stimulating Hormone, Glucagon, Glucagon-like Peptide 1, Glutathione S-Transferase alpha, Granulocyte Colony-Stimulating Factor, Granulocyte-Macrophage Colony-Stimulating Factor, Growth Hormone, Growth-Regulated alpha protein, Haptoglobin, Heat Shock Protein 60, Heparin-Binding EGF-Like Growth Factor, Hepatocyte Growth Factor, Immunoglobulin A, Immunoglobulin E, Immunoglobulin M, Insulin, Insulin-like Growth Factor I, Insulin-like Growth Factor-Binding Protein 2, Intercellular Adhesion Molecule 1, Interferon gamma, Interferon gamma Induced Protein 10, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-6 Receptor, Interleukin-7, Interleukin-8, Interleukin-10, Interleukin-11, Interleukin-12 Subunitp40, Interleukin-12 Subunitp70, Interleukin-13, Interleukin-15, Interleukin-16, Interleukin-25, Kidney Injury Molecule-1, Lectin-Like Oxidized LDL Receptor 1, Leptin, Luteinizing Hormone, Lymphotactin, Macrophage Colony-Stimulating Factor 1, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Macrophage Inflammatory Protein-3 alpha, Macrophage Migration Inhibitory Factor, Macrophage-Derived Chemokine, Malondialdehyde-Modified Low-Density Lipoprotein, Matrix Metalloproteinase-1, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-7, Matrix Metalloproteinase-9, Matrix Metalloproteinase-9, Matrix Metalloproteinase-10, Monocyte Chemotactic Protein 1, Monocyte Chemotactic Protein 2, Monocyte Chemotactic Protein 3, Monocyte Chemotactic Protein 4, Monokine Induced by Gamma Interferon, Myeloid Progenitor Inhibitory Factor 1, Myeloperoxidase, Myoglobin, Nerve Growth Factor beta, Neuronal Cell Adhesion Molecule, Neutrophil Gelatinase-Associated Lipocalin, NT-proBNP, Osteopontin, Pancreatic Polypeptide, Peptide YY, Placenta Growth Factor, Plasminogen Activator Inhibitor 1, Platelet-Derived Growth Factor BB, Pregnancy-Associated Plasma Protein A, Progesterone, Proinsulin (inc. Intact or Total), Prolactin, Prostate-Specific Antigen (inc. Free PSA), Prostatic Acid Phosphatase, Pulmonary and Activation-Regulated Chemokine, Receptor for advanced glycosylation end products, Resistin, S100 calcium-binding protein B, Secretin, Serotransferrin, Serum Amyloid P-Component, Serum Glutamic Oxaloacetic Transaminase, Sex Hormone-Binding Globulin, Sortilin, Stem Cell Factor, Superoxide Dismutase 1 (soluble), T Lymphocyte-Secreted Protein 1-309, Tamm-Horsfall Urinary Glycoprotein, T-Cell-Specific Protein RANTES, Tenascin-C, Testosterone, Thrombomodulin, Thrombopoietin, Thrombospondin-1, Thyroid-Stimulating

PREOMICS EXHIBIT 1002
Page 1038 of 1861

	Hormone, Thyroxine-Binding Globulin, Tissue Factor, Tissue Inhibitor of Metalloproteinases 1, TNF-Related Apoptosis-Inducing Ligand Receptor 3, Transforming Growth Factor alpha, Transforming Growth Factor beta-3, Transthyretin, Trefoil Factor 3, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Tumor necrosis Factor Receptor 2, Vascular Cell Adhesion Molecule-1, Vascular Endothelial Growth Factor, Vitamin K-Dependent Protein S, Vitronectin, von Willebrand Factor
Oncology	6Ckine, Aldose Reductase, Alpha-Fetoprotein, Amphiregulin, Angiogenin, Annexin A1, B cell-activating Factor, B Lymphocyte Chemoattractant, Bcl-2-like protein 2, Betacellulin, Cancer Antigen 125, Cancer Antigen 15-3, Cancer Antigen 19-9, Cancer Antigen 72-4, Carcinoembryonic Antigen, Cathepsin D, Cellular Fibronectin, Collagen IV, Endoglin, Endostatin, Eotaxin-2, Epidermal Growth Factor, Epiregulin, Epithelial cell adhesion molecule, Ezrin, Fatty Acid-Binding Protein (adipocyte), Fatty Acid-Binding Protein (liver), Fibroblast Growth Factor basic, Fibulin-1C, Galectin-3, Gelsolin, Glucose-6-phosphate Isomerase, Glutamate-Cysteine Ligase Regulatory subunit, Glutathione S-Transferase Mu 1, HE4, Heparin-Binding EGF-Like Growth Factor, Hepatocyte Growth Factor, Hepatocyte Growth Factor Receptor, Hepsin, Human Chorionic Gonadotropin beta, Human Epidermal Growth Factor Receptor 2, Insulin-like Growth Factor-Binding Protein 1, Insulin-like Growth Factor-Binding Protein 2, Insulin-like Growth Factor-Binding Protein 3, Insulin-like Growth Factor Binding Protein 4, Insulin-like Growth Factor Binding Protein 5, Insulin-like Growth Factor Binding Protein 6, Interferon gamma Induced Protein 10, Interferon-inducible T-cell alpha chemoattractant, Interleukin-2 Receptor alpha, Interleukin-6, Interleukin-6 Receptor subunit beta, Kallikrein 5, Kallikrein-7, Lactoylglutathione lyase, Latency-Associated Peptide of Transforming Growth Factor beta 1, Leptin, Macrophage inflammatory protein 3 beta, Macrophage Migration Inhibitory Factor, Macrophage-Stimulating Protein, Maspin, Matrix Metalloproteinase-2, Mesothelin, MHC class I chain-related protein A, Monocyte Chemotactic Protein 1, Monokine Induced by Gamma Interferon, Neuron-Specific Enolase, Neuropilin-1, Neutrophil Gelatinase-Associated Lipocalin, Nucleoside diphosphate kinase B, Osteopontin, Osteoprotegerin, Pepsinogen I, Peroxiredoxin-4, Phosphoserine Aminotransferase, Placenta Growth Factor, Platelet-Derived Growth Factor BB, Prostasin, Protein S100-A4, Protein S100-A6, Receptor tyrosine-protein kinase erbB-3, Squamous Cell Carcinoma Antigen-1, Stromal cell-derived Factor-1, Tenascin-C, Tetranectin, Thyroglobulin, Tissue type Plasminogen activator, Transforming Growth Factor alpha, Tumor Necrosis Factor Receptor I, Tyrosine kinase with Ig and EGF homology domains 2, Urokinase-type Plasminogen Activator, Urokinase-type plasminogen activator Receptor, Vascular Endothelial Growth Factor, Vascular endothelial growth Factor B, Vascular Endothelial Growth Factor C, Vascular endothelial growth Factor D, Vascular Endothelial Growth Factor Receptor 1, Vascular Endothelial Growth Factor Receptor 2, Vascular endothelial growth Factor Receptor 3, YKL-40
Disease	Adiponectin, Alpha-1-Antitrypsin, Alpha-2-Macroglobulin, Alpha-Fetoprotein, Apolipoprotein A-I, Apolipoprotein C-III, Apolipoprotein H, Apolipoprotein(a), Beta-2-Microglobulin, Brain-Derived Neurotrophic Factor, Calcitonin, Cancer Antigen 125, Cancer Antigen 19-9, Carcinoembryonic Antigen, CD 40 antigen, CD40 Ligand, Complement C3, C-Reactive Protein, Creatine Kinase-MB, Endothelin-1, EN-RAGE, Eotaxin-1, Epidermal Growth Factor, Epithelial-Derived Neutrophil-Activating Protein 78, Erythropoietin, Factor VII, Fatty Acid-Binding Protein (heart), Ferritin, Fibrinogen, Fibroblast Growth Factor basic, Granulocyte Colony-Stimulating Factor, Granulocyte-Macrophage Colony-Stimulating Factor, Growth Hormone, Haptoglobin, Immunoglobulin A, Immunoglobulin E, Immunoglobulin M, Insulin, Insulin-like Growth Factor I, Intercellular Adhesion Molecule 1, Interferon gamma, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-7, Interleukin-8, Interleukin-10, Interleukin-12 Subunit p40, Interleukin-12 Subunit p70, Interleukin-13, Interleukin-15, Interleukin-16, Leptin, Lymphotactin, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta,

PREOMICS EXHIBIT 1002
Page 1039 of 1861

	Macrophage-Derived Chemokine, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-9, Monocyte Chemotactic Protein 1, Myeloperoxidase, Myoglobin, Plasminogen Activator Inhibitor 1, Pregnancy-Associated Plasma Protein A, Prostate-Specific Antigen (inc. Free PSA), Prostatic Acid Phosphatase, Serum Amyloid P-Component, Serum Glutamic Oxaloacetic Transaminase, Sex Hormone-Binding Globulin, Stem Cell Factor, T-Cell-Specific Protein RANTES, Thrombopoietin, Thyroid-Stimulating Hormone, Thyroxine-Binding Globulin, Tissue Factor, Tissue Inhibitor of Metalloproteinases 1, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Tumor Necrosis Factor Receptor 2, Vascular Cell Adhesion Molecule-1, Vascular Endothelial Growth Factor, von Willebrand Factor
Neurological	Alpha-1-Antitrypsin, Apolipoprotein A-I, Apolipoprotein A-II, Apolipoprotein B, Apolipoprotein C-I, Apolipoprotein H, Beta-2-Microglobulin, Betacellulin, Brain-Derived Neurotrophic Factor, Calbindin, Cancer Antigen 125, Carcinoembryonic Antigen, CD5 Antigen-like, Complement C3, Connective Tissue Growth Factor, Cortisol, Endothelin-1, Epidermal Growth Factor Receptor, Ferritin, Fetuin-A, Follicle-Stimulating Hormone, Haptoglobin, Immunoglobulin A, Immunoglobulin M, Intercellular Adhesion Molecule 1, Interleukin-6 Receptor, Interleukin-7, Interleukin-10, Interleukin-11, Interleukin-17, Kidney Injury Molecule-1, Luteinizing Hormone, Macrophage-Derived Chemokine, Macrophage Migration Inhibitory Factor, Macrophage Inflammatory Protein-1 alpha, Matrix Metalloproteinase-2, Monocyte Chemotactic Protein 2, Peptide YY, Prolactin, Prostatic Acid Phosphatase, Serotransferrin, Serum Amyloid P-Component, Sortilin, Testosterone, Thrombopoietin, Thyroid-Stimulating Hormone, Tissue Inhibitor of Metalloproteinases 1, TNF-Related Apoptosis-Inducing Ligand Receptor 3, Tumor necrosis Factor Receptor 2, Vascular Endothelial Growth Factor, Vitronectin
Cardiovascular	Adiponectin, Apolipoprotein A-I, Apolipoprotein B, Apolipoprotein C-III, Apolipoprotein D, Apolipoprotein E, Apolipoprotein H, Apolipoprotein(a), Clusterin, C-Reactive Protein, Cystatin-C, EN-RAGE, E-Selectin, Fatty Acid-Binding Protein (heart), Ferritin, Fibrinogen, Haptoglobin, Immunoglobulin M, Intercellular Adhesion Molecule 1, Interleukin-6, Interleukin-8, Lectin-Like Oxidized LDL Receptor 1, Leptin, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Malondialdehyde-Modified Low-Density Lipoprotein, Matrix Metalloproteinase-1, Matrix Metalloproteinase-10, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-7, Matrix Metalloproteinase-9, Monocyte Chemotactic Protein 1, Myeloperoxidase, Myoglobin, NT-proBNP, Osteopontin, Plasminogen Activator Inhibitor 1, P-Selectin, Receptor for advanced glycosylation end products, Serum Amyloid P-Component, Sex Hormone-Binding Globulin, T-Cell-Specific Protein RANTES, Thrombomodulin, Thyroxine-Binding Globulin, Tissue Inhibitor of Metalloproteinases 1, Tumor Necrosis Factor alpha, Tumor necrosis Factor Receptor 2, Vascular Cell Adhesion Molecule-1, von Willebrand Factor
Inflammatory	Alpha-1-Antitrypsin, Alpha-2-Macroglobulin, Beta-2-Microglobulin, Brain-Derived Neurotrophic Factor, Complement C3, C-Reactive Protein, Eotaxin-1, Factor VII, Ferritin, Fibrinogen, Granulocyte-Macrophage Colony-Stimulating Factor, Haptoglobin, Intercellular Adhesion Molecule 1, Interferon gamma, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-7, Interleukin-8, Interleukin-10, Interleukin-12 Subunitp40, Interleukin-12 Subunitp70, Interleukin-15, Interleukin-17, Interleukin-23, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-9, Monocyte Chemotactic Protein 1, Stem Cell Factor, T-Cell-Specific Protein RANTES, Tissue Inhibitor of Metalloproteinases 1, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Tumor necrosis Factor Receptor 2, Vascular Cell Adhesion Molecule-1, Vascular Endothelial Growth Factor, Vitamin D-Binding Protein, von Willebrand Factor
Metabolic	Adiponectin, Adrenocorticotropic Hormone, Angiotensin-Converting Enzyme,

PREOMICS EXHIBIT 1002
Page 1040 of 1861

	Angiotensinogen, Complement C3 alpha des arg, Cortisol, Follicle-Stimulating Hormone, Galanin, Glucagon, Glucagon-like Peptide 1, Insulin, Insulin-like Growth Factor I, Leptin, Luteinizing Hormone, Pancreatic Polypeptide, Peptide YY, Progesterone, Prolactin, Resistin, Secretin, Testosterone
Kidney	Alpha-1-Microglobulin, Beta-2-Microglobulin, Calbindin, Clusterin, Connective Tissue Growth Factor, Creatinine, Cystatin-C, Glutathione S-Transferase alpha, Kidney Injury Molecule-1, Microalbumin, Neutrophil Gelatinase-Associated Lipocalin, Osteopontin, Tamm-Horsfall Urinary Glycoprotein, Tissue Inhibitor of Metalloproteinases 1, Trefoil Factor 3, Vascular Endothelial Growth Factor
Cytokines	Granulocyte-Macrophage Colony-Stimulating Factor, Interferon gamma, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-7, Interleukin-8, Interleukin-10, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Matrix Metalloproteinase-2, Monocyte Chemotactic Protein 1, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Brain-Derived Neurotrophic Factor, Eotaxin-1, Intercellular Adhesion Molecule 1, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-12 Subunitp40, Interleukin-12 Subunitp70, Interleukin-15, Interleukin-17, Interleukin-23, Matrix Metalloproteinase-3, Stem Cell Factor, Vascular Endothelial Growth Factor
Protein	14.3.3 gamma, 14.3.3 (Pan), 14-3-3 beta, 6-Histidine, a-B-Crystallin, Acinus, Actin beta, Actin (Muscle Specific), Actin (Pan), Actin (skeletal muscle), Activin Receptor Type II, Adenovirus, Adenovirus Fiber, Adenovirus Type 2 E1A, Adenovirus Type 5 E1A, ADP-ribosylation Factor (ARF-6), Adrenocorticotrophic Hormone, AIF (Apoptosis Inducing Factor), Alkaline Phosphatase (AP), Alpha Fetoprotein (AFP), Alpha Lactalbumin, alpha-1-antichymotrypsin, alpha-1-antitrypsin, Amphiregulin, Amylin Peptide, Amyloid A, Amyloid A4 Protein Precursor, Amyloid Beta (APP), Androgen Receptor, Ang-1, Ang-2, APC, APC1 1, APC2, Apolipoprotein D, A-Raf, ARC, Askl / MAPKKK5, ATM, Axonal Growth Cones, b Galactosidase, b-2-Microglobulin, B7-H2, BAG-1, Bak, Bax, B-Cell, B-cell Linker Protein (BLNK), Bel 10 / CIPER / CLAP / mElO, bcl-2a, Bcl-6, bcl-X, bcl-XL, Bim (BOD), Biotin, Bonzo / STRL33 / TYMSTR, Bovine Serum Albumin, BRCA2 (aa 1323-1346), BrdU, Bromodeoxyuridine (BrdU), CA125, CA19-9, c-Abl, Cadherin (Pan), Cadherin-E, Cadherin-P, Calcitonin, Calcium Pump ATPase, Caldesmon, Calmodulin, Calponin, Calretinin, Casein, Caspase 1, Caspase 2, Caspase 3, Caspase 5, Caspase 6 (Mch 2), Caspase 7 (Mch 3), Caspase 8 (FLICE), Caspase 9, Catenin alpha, Catenin beta, Catenin gamma, Cathepsin D, CCK-8, CD1, CD10, CDIOO/Leukocyte Semaphorin, CD105, CD106 / VCAM, CD1 15/c-fms/CSF-lR/M-CSFR, CD137 (4-1BB), CD138, CD14, CD15, CD155/PVR (Polio Virus Receptor), CD16, CD165, CD18, CDla, CDlb, CD2, CD20, CD21, CD23, CD231, CD24, CD25/IL-2 Receptor a, CD26/DPP IV, CD29, CD30 (Reed-Sternberg Cell Marker), CD32/Fcg Receptor II, CD35/CR1, CD36GPIIIb/GPIV, CD3zeta, CD4, CD40, CD42b, CD43, CD45/T200/LCA, CD45RB, CD45RO, CD46, CD5, CD50/ICAM-3, CD53, CD54/ICAM-1, CD56/NCAM-1, CD57, CD59 / MACIF / MIRL / Protectin, CD6, CD61 / Platelet Glycoprotein IIIA, CD63, CD68, CD71 / Transferrin Receptor, CD79amb-l, CD79b, CD8, CD81/TAPA-1, CD84, CD9, CD94, CD95 / Fas, CD98, CDC14A Phosphatase, CDC25C, CDC34, CDC37, CDC47, CDC6, cdhl, Cdkl/p34cdc2, Cdk2, Cdk3, Cdk4, Cdk5, Cdk7, Cdk8, CDwl7, CDw60, CDw75, CDw78, CEA/CD66e, c-erbB-2/HER-2/neu Ab-1 (21N), c-erbB-4/HER-4, c-fos, Chkl, Chorionic Gonadotropin beta (hCG-beta), Chromogranin A, CIDE-A, CIDE-B, CITED 1, c-jun, Clathrin, claudin 11, Claudin 2, Claudin 3, Claudin 4, Claudin 5, CLAUDIN 7, Claudin-1, CNPase, Collagen II, Collagen IV, Collagen IX, Collagen VII, Connexin 43, COX2, CREB, CREB-Binding Protein, Cryptococcus neoformans, c-Src, Cullin-1 (CUL-1), Cullin-2 (CUL-2), Cullin-3 (CUL-3), CXCR4 / Fusin, Cyclin Bl, Cyclin C, Cyclin Dl, Cyclin D3, Cyclin E, Cyclin E2, Cystic Fibrosis Transmembrane Regulator, Cytochrome c, D4-GDI, Daxx, DcRl, DcR2 / TRAIL-R4 / TRUNDD, Desmin, DFF40 (DNA Fragmentation Factor 40) / CAD, DFF45 / ICAD, DJ-1, DNA Ligase I, DNA Polymerase Beta, DNA Polymerase Gamma, DNA Primase (p49), DNA Primase (p58), DNA-PKcs, DP-2, DR3, DR5, Dysferlin, Dystrophin, E2F-1, E2F-2, E2F-3,

PREOMICS EXHIBIT 1002
Page 1041 of 1861

	E2F-4, E2F-5, E3-binding protein (ARM1), EGFR, EMA/CA15-3/MUC-1, Endostatin, Epithelial Membrane Antigen (EMA / CA15-3 / MUC-1), Epithelial Specific Antigen, ER beta, ER Ca+2 ATPase2, ERCC1, Erkl, ERK2, Estradiol, Estriol, Estrogen Receptor, Exol, Ezrin/p81/80K/Cytovillin, F.VIII/VWF, Factor VIII Related Antigen, FADD (FAS-Associated death domain-containing protein), Fascin, Fas-ligand, Ferritin, FGF-1, FGF-2, FHIT, Fibrillin-1, Fibronectin, Filaggrin, Filamin, FITC, Fli-1, FLIP, Flk-1 / KDR / VEGFR2, Flt-1 / VEGFR1, Flt-4, Fra2, FSH, FSH-b, Fyn, GaO, Gab-1, GABA a Receptor 1, GAD65, Gail, Gamma Glutamyl Transferase (gGT), Gamma Glutamylcysteine Synthetase(GCS)/Glutamate-cysteine Ligase, GAPDH, Gastrin 1, GCDFP-15, G-CSF, GFAP, Glicentin, Glucagon, Glucose-Regulated Protein 94, GluR 2/3, GluRl, GluR4, GluR6/7, GLUT-1, GLUT-3, Glycogen Synthase Kinase 3b (GSK3b), Glycophorin A, GM-CSF, GnRH Receptor, Golgi Complex, Granulocyte, Granzyme B, Grb2, Green Fluorescent Protein (GFP), GRIPl, Growth Hormone (hGH), GSK-3, GST, GSTmu, H.Pylori, HDAC1, HDJ-2/DNAJ, Heat Shock Factor 1, Heat Shock Factor 2, Heat Shock Protein 27/hsp27, Heat Shock Protein 60/hsp60, Heat Shock Protein 70/hsp70, Heat Shock Protein 75/hsp75, Heat Shock Protein 90a/hsp86, Heat Shock Protein 90b/hsp84, Helicobacter pylori, Heparan Sulfate Proteoglycan, Hepatic Nuclear Factor-3B, Hepatocyte, Hepatocyte Factor Homologue-4, Hepatocyte Growth Factor, Heregulin, HIF-la, Histone HI, hPL, HPV 16, HPV 16-E7, HRP, Human Sodium Iodide Symporter (hNIS), I-FLICE / CASPER, IFN gamma, IgA, IGF-1R, IGF-I, IgG, IgM (m-Heavy Chain), I-Kappa-B Kinase b (IKKb), IL-1 alpha, IL-1 beta, IL-10, IL-10R, IL17, IL-2, IL-3, IL-30, IL-4, IL-5, IL-6, IL-8, Inhibin alpha, Insulin, Insulin Receptor, Insulin Receptor Substrate-1, Int-2 Oncoprotein, Integrin beta5, Interferon-a(II), Interferon-g, Involucrin, IP10/CRG2, IPO-38 Proliferation Marker, IRAK, ITK, JNK Activating kinase (JKK1), Kappa Light Chain, Keratin 10, Keratin 10/13, Keratin 14, Keratin 15, Keratin 16, Keratin 18, Keratin 19, Keratin 20, Keratin 5/6/18, Keratin 5/8, Keratin 8, Keratin 8 (phospho-specific Ser73), Keratin 8/18, Keratin (LMW), Keratin (Multi), Keratin (Pan), Ki67, Ku (p70/p80), Ku (p80), LI Cell Adhesion Molecule, Lambda Light Chain, Laminin Bl/bl, Laminin B2/gl, Laminin Receptor, Laminin-s, Lck, Lck (p561ck), Leukotriene (C4, D4, E4), LewisA, LewisB, LH, L-Plastin, LRP / MVP, Luciferase, Macrophage, MADD, MAGE-1, Maltose Binding Protein, MAP IB, MAP2a,b, MART-1/Melan-A, Mast Cell Chymase, Mcl-1, MCM2, MCM5, MDM2, Medroxyprogesterone Acetate (MPA), Mekl, Mek2, Mek6, Mekk-1, Melanoma (gplOO), mGluRl, mGluR5, MGMT, MHC I (HLA25 and HLA-Aw32), MHC I (HLA-A), MHC I (HLA-A,B,C), MHC I (HLA-B), MHC II (HLA-DP and DR), MHC II (HLA-DP), MHC II (HLA-DQ), MHC II (HLA-DR), MHC II (HLA-DR) la, Microphthalmia, Milk Fat Globule Membrane Protein, Mitochondria, MLH1, MMP-1 (Collagenase-I), MMP-10 (Stromilysin-2), MMP-11 (Stromelysin-3), MMP-13 (Collagenase-3), MMP-14 / MT1-MMP, MMP-15 / MT2-MMP, MMP-16 / MT3-MMP, MMP-19, MMP-2 (72kDa Collagenase IV), MMP-23, MMP-7 (Matrilysin), MMP-9 (92kDa Collagenase IV), Moesin, mRANKL, Muc-1, Mucin 2, Mucin 3 (MUC3), Mucin 5AC, MyD88, Myelin / Oligodendrocyte, Myeloid Specific Marker, Myeloperoxidase, MyoDl, Myogenin, Myoglobin, Myosin Smooth Muscle Heavy Chain, Nek, Negative Control for Mouse IgGl, Negative Control for Mouse IgG2a, Negative Control for Mouse IgG3, Negative Control for Mouse IgM, Negative Control for Rabbit IgG, Neurofilament, Neurofilament (160kDa), Neurofilament (200kDa), Neurofilament (68kDa), Neuron Specific Enolase, Neutrophil Elastase, NF kappa B / p50, NF kappa B / p65 (Rel A), NGF-Receptor (p75NGFR), brain Nitric Oxide Synthase (bNOS), endothelial Nitric Oxide Synthase (eNOS), nm23, NOS-i, NOS-u, Notch, Nucleophosmin (NPM), NuMA, O ct-1, Oct-2/, Oct-3/, Ornithine Decarboxylase, Osteopontin, p i 30, pl30cas, pl4ARF, pl5INK4b, pl6INK4a, p i 70, p i 70 / MDR-1, pl8INK4c, pl9ARF, pl9Skpl, p21WAFl, p27Kipl, p300 / CBP, $_{P}$35nck5a, P504S, p53, p57Kip2 Ab-7, p63 (p53 Family Member), p73, p73a, p73a/b, p95VAV, Parathyroid Hormone, Parathyroid Hormone Receptor Type 1, Parkin, PARP, PARP (Poly ADP-Ribose Polymerase), Pax-5, Paxillin, PCNA, PCTAIRE2, PDGF, PDGFR alpha, PDGFRbeta, Pdsl, Perforin, PGP9.5, PHAS-

PREOMICS EXHIBIT 1002
Page 1042 of 1861

	I, PHAS-II, Phospho-Ser/Thr/Tyr, Phosphotyrosine, PLAP, Plasma Cell Marker, Plasminogen, PLC gamma 1, PMP-22, Pneumocystis jiroveci, PPAR-gamma, PR3 (Proteinase 3), Presenillin, Progesterone, Progesterone Receptor, Progesterone Receptor (phospho-specific) - Serine 190, Progesterone Receptor (phospho-specific) - Serine 294, Prohibitin, Prolactin, Prolactin Receptor, Prostate Apoptosis Response Protein-4, Prostate Specific Acid Phosphatase, Prostate Specific Antigen, pS2, PSCA, Rabies Virus, RAD1, Rad51, Rafl, Raf-1 (Phospho-specific), RAIDD, Ras, Radl8, Renal Cell Carcinoma, Ret Oncoprotein, Retinoblastoma, Retinoblastoma (Rb) (Phospho-specific Serine608), Retinoic Acid Receptor (b), Retinoid X Receptor (hRXR), Retinol Binding Protein, Rhodopsin (Opsin), ROC, RPA/p32, RPA/p70, Ruv A, Ruv B, Ruv C, S100, S100A4, S100A6, SHP-1, SIM Ag (SIMA-4D3), SIRP al, sm, SODD (Silencer of Death Domain), Somatostatin Receptor-I, SRC1 (Steroid Receptor Coactivator-1) Ab-1, SREBP-1 (Sterol Regulatory Element Binding Protein- 1), SRF (Serum Response Factor), Stat-1, Stat3, Stat5, Stat5a, Stat5b, Stat6, Streptavidin, Superoxide Dismutase, Surfactant Protein A, Surfactant Protein B, Surfactant Protein B (Pro), Survivin, SV40 Large T Antigen, Syk, Synaptophysin, Synuclein, Synuclein beta, Synuclein pan, TACE (TNF-alpha converting enzyme) / ADAM 17, TAG-72, tau, TdT, Tenascin, Testosterone, TGF beta 3, TGF-beta 2, Thomsen-Friedenreich Antigen, Thrombospondin, Thymidine Phosphorylase, Thymidylate Synthase, Thymine Glycols, Thyroglobulin, Thyroid Hormone Receptor beta, Thyroid Hormone Receptor, Thyroid Stimulating Hormone (TSH), TID-1, TIMP-1, TIMP-2, TNF alpha, TNFa, TNR-R2, Topo II beta, Topoisomerase Ila, Toxoplasma Gondii, TR2, TRADD, Transforming Growth Factor a, Transglutaminase II, TRAP, Tropomyosin, TRP75 / gp75, TrxR2, TTF-1, Tubulin, Tubulin-a, Tubulin-b, Tyrosinase, Ubiquitin, UCP3, uPA, Urocortin, Vacular Endothelial Growth Factor(VEGF), Vimentin, Vinculin, Vitamin D Receptor (VDR), von Hippel-Lindau Protein, Wnt-1, Xanthine Oxidase, XPA, XPF, XPG, XRCC1, XRCC2, ZAP-70, Zip kinase
Known Cancer Genes	ABL1, ABL2, ACSL3, AF15Q14, AF1Q, AF3p21, AF5q31, AKAP9, AKT1, AKT2, ALDH2, ALK, AL017, APC, ARHGEF12, ARHH, ARID 1A, ARID2, ARNT, ASPSCR1, ASXL1, ATF1, ATIC, ATM, ATRX, BAP1, BCL10, BCL1 1A, BCL1 IB, BCL2, BCL3, BCL5, BCL6, BCL7A, BCL9, BCOR, BCR, BHD, BIRC3, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BRD3, BRD4, BRIP1, BTG1, BUB IB, C12orf9, C15orf21, C15orf55, C16orf75, CANT1, CARD11, CARS, CBFA2T1, CBFA2T3, CBFB, CBL, CBLB, CBLC, CCNB1IP1, CCND1, CCND2, CCND3, CCNE1, CD273, CD274, CD74, CD79A, CD79B, CDH1, CDH11, CDK12, CDK4, CDK6, CDKN2A, CDKN2a(pl4), CDKN2C, CDX2, CEBPA, CEP1, CHCHD7, CHEK2, CHIC2, CHN1, CIC, CIITA, CLTC, CLTCL1, CMKOR1, COL1A1, COPEB, COX6C, CREB1, CREB3L1, CREB3L2, CREBBP, CRLF2, CRTC3, CTNNB1, CYLD, D10S170, DAXX, DDB2, DDIT3, DDX10, DDX5, DDX6, DEK, DICERl, DNMT3A, DUX4, EBF1, EGFR, EIF4A2, ELF4, ELK4, ELKS, ELL, ELN, EML4, EP300, EPS 15, ERBB2, ERCC2, ERCC3, ERCC4, ERCC5, ERG, ETV1, ETV4, ETV5, ETV6, EVI1, EWSR1, EXT1, EXT2, EZH2, FACL6, FAM22A, FAM22B, FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FBXOl 1, FBXW7, FCGR2B, FEV, FGFR1, FGFRIOP, FGFR2, FGFR3, FH, FHIT, FIP1L1, FLU, FLJ27352, FLT3, FNBP1, FOXL2, FOXOIA, FOX03A, FOXP1, FSTL3, FUBPl, FUS, FVTl, GAS7, GATAl, GATA2, GATA3, GMPS, GNAll, GNAQ, GNAS, GOLGA5, GOPC, GPC3, GPHN, GRAF, HCMOGT-1, HEAB, HERPUD1, HEY1, HIP1, HIST1H4I, HLF, HLXB9, HMGA1, HMGA2, HNRNPA2B1, HOOK3, HOXA11, HOXA13, HOXA9, HOXC11, HOXC13, HOXD11, HOXD13, HRAS, HRPT2, HSPCA, HSPCB, IDH1, IDH2, IGH@, IGK@, IGL@, IKZF1, IL2, IL21R, IL6ST, IL7R, IRF4, IRTA1, ITK, JAK1, JAK2, JAK3, JAZF1, JUN, KDM5A, KDM5C, KDM6A, KDR, KIAA1549, KIT, KLK2, KRAS, KTN1, LAF4, LASP1, LCK, LCP1, LCX, LHFP, LIFR, LMOl, LM02, LPP, LYL1, MADH4, MAF, MAFB, MALT1, MAML2, MAP2K4, MDM2, MDM4, MDS1, MDS2, MECT1, MED 12, MEN1, MET, MITF, MKL1, MLF1, MLH1, MLL, MLL2, MLL3, MLLT1, MLLT10, MLLT2, MLLT3, MLLT4, MLLT6, MLLT7, MN1, MPL, MSF, MSH2, MSH6, MSI2, MSN,

PREOMICS EXHIBIT 1002
Page 1043 of 1861

	MTCP1, MUC1, MUTYH, MYB, MYC, MYCLl, MYCN, MYD88, MYH11, MYH9, MYST4, NACA, NBS1, NCOA1, NCOA2, NCOA4, NDRG1, NF1, NF2, NFE2L2, NFIB, NFKB2, NIN, NKX2-1, NONO, NOTCH 1, NOTCH2, NPM1, NR4A3, NRAS, NSD1, NTRK1, NTRK3, NUMA1, NUP214, NUP98, OLIG2, OMD, P2RY8, PAFAH1B2, PALB2, PAX3, PAX5, PAX7, PAX8, PBRM1, PBX1, PCM1, PCSK7, PDE4DIP, PDGFB, PDGFRA, PDGFRB, PERI, PHOX2B, PICALM, PIK3CA, PIK3R1, PIM1, PLAG1, PML, PMS1, PMS2, PMX1, PNUTL1, POU2AF1, POU5F1, PPARG, PPP2R1A, PRCC, PRDM1, PRDM16, PRF1, PRKARIA, PRO1073, PSIP2, PTCH, PTEN, PTPN11, RAB5EP, RAD51L1, RAFl, RALGDS, RANBP17, RAP1GDS1, RARA, RBI, RBM15, RECQL4, REL, RET, ROS1, RPL22, RPN1, RUNDC2A, RUNX1, RUNXBP2, SBDS, SDH5, SDHB, SDHC, SDHD, SEPT6, SET, SETD2, SF3B1, SFPQ, SFRS3, SH3GL1, SIL, SLC45A3, SMARCA4, SMARCB1, SMO, SOCS1, SOX2, SRGAP3, SRSF2, SS18, SS18L1, SSH3BP1, SSX1, SSX2, SSX4, STK11, STL, SUFU, SUZ12, SYK, TAF15, TALI, TAL2, TCEA1, TCF1, TCF12, TCF3, TCF7L2, TCLIA, TCL6, TET2, TFE3, TFEB, TFG, TFPT, TFRC, THRAP3, TIF1, TLX1, TLX3, TMPRSS2, TNFAIP3, TNFRSF14, TNFRSF17, TNFRSF6, TOPI, TP53, TPM3, TPM4, TPR, TRA@, TRB@, TRD@, TRIM27, TRIM33, TRIP1 1, TSC1, TSC2, TSHR, TTL, U2AF1, USP6, VHL, VTI1A, WAS, WHSC1, WHSC1L1, WIF1, WRN, WT1, WTX, XPA, XPC, XPOl, YWHAE, ZNF145, ZNF198, ZNF278, ZNF331, ZNF384, ZNF521, ZNF9, ZRSR2
Known Cancer Genes	AR, androgen receptor; ARPC1A, actin-related protein complex 2/3 subunit A; AURKA, Aurora kinase A; BAG4, BCl-2 associated anthogene 4; BC1212, BCl-2 like 2; BIRC2, Baculovirus IAP repeat containing protein 2; CACNA1E, calcium channel voltage dependent alpha-IE subunit; CCNE1, cyclin El; CDK4, cyclin dependent kinase 4; CHD1L, chromodomain helicase DNA binding domain 1-like; CKS1B, CDC28 protein kinase IB; COPS3, COP9 subunit 3; DCUN1D1, DCN1 domain containing protein 1; DYRK2, dual specificity tyrosine phosphorylation regulated kinase 2; EEF1A2, eukaryotic elongation transcription factor 1 alpha 2; EGFR, epidermal growth factor receptor; FADD, Fas-associated via death domain; FGFR1, fibroblast growth factor receptor 1, GATA6, GATA binding protein 6; GPC5, glypican 5; GRB7, growth factor receptor bound protein 7; MAP3K5, mitogen activated protein kinase kinase kinase 5; MED29, mediator complex subunit 5; MITF, microphthalmia associated transcription factor; MTDH, metadherin; NCOA3, nuclear receptor coactivator 3; NKX2-1, NK2 homeobox 1; PAKl, p21/CDC42/RACl-activated kinase 1; PAX9, paired box gene 9; PIK3CA, phosphatidylinositol-3 kinase catalytic a; PLA2G10, phopholipase A2, group X; PPM1D, protein phosphatase magnesium-dependent ID; PTK6, protein tyrosine kinase 6; PRKCI, protein kinase C iota; RPS6KB1, ribosomal protein s6 kinase 70kDa; SKP2, s-phase kinase associated protein; SMURF1, sMAD specific E3 ubiquitin protein ligase 1; SHH, sonic hedgehog homologue; STARD3, sTAR-related lipid transfer domain containing protein 3; YWHAQ, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta isoform; ZNF217, zinc finger protein 217
Mitotic Related Cancer Genes	Aurora kinase A (AURKA); Aurora kinase B (AURKB); Baculoviral IAP repeat-containing 5, survivin (BIRC5); Budding uninhibited by benzimidazoles 1 homolog (BUB1); Budding uninhibited by benzimidazoles 1 homolog beta, BUBR1 (BUB IB); Budding uninhibited by benzimidazoles 3 homolog (BUB3); CDC28 protein kinase regulatory subunit IB (CKS1B); CDC28 protein kinase regulatory subunit 2 (CKS2); Cell division cycle 2 (CDC2)/CDK1 Cell division cycle 20 homolog (CDC20); Cell division cycle-associated 8, borealin (CDCA8); Centromere protein F, mitosin (CENPF); Centrosomal protein 110 kDa (CEP 110); Checkpoint with forkhead and ring finger domains (CHFR); Cyclin B1 (CCNB1); Cyclin B2 (CCNB2); Cytoskeleton-associated protein 5 (CKAP5/ch-TOG); Microtubule-associated protein RP/ EB family member 1. End-binding protein 1, EB1 (MAPRE1); Epithelial cell transforming sequence 2 oncogene (ECT2); Extra spindle poles like 1, separase (ESPL1); Forkhead box M1 (FOXM1); H2A histone family, member X (H2AFX); Kinesin family member 4A (KIF4A); Kinetochore-associated 1 (KNTC1/ROD); Kinetochore-associated 2; highly expressed in

PREOMICS EXHIBIT 1002
Page 1044 of 1861

	cancer 1 (KNTC2/HEC1); Large tumor suppressor, homolog 1 (LATS1); Large tumor suppressor, homolog 2 (LATS2); Mitotic arrest deficient-like 1; MAD1 (MADILI); Mitotic arrest deficient-like 2; MAD2 (MAD2L1); Mpsl protein kinase (TTK); Never in mitosis gene a-related kinase 2 (NEK2); Ninein, GSK3b interacting protein (NEST); Non-SMC condensin I complex, subunit D2 (NCAPD2/CNAP 1); Non-SMC condensin I complex, subunit H (NACPH/CAPH); Nuclear mitotic apparatus protein 1 (NUMA1); Nucleophosmin (nucleolar phosphoprotein B23, numatrin); (NPM1); Nucleoporin (NUP98); Pericentriolar material 1 (PCM1); Pituitary tumor-transforming 1, securin (PTTG1); Polo-like kinase 1 (PLK1); Polo-like kinase 4 (PLK4/SAK); Protein (peptidylprolyl cis/trans isomerase) NIMA-interacting 1 (PEMl); Protein regulator of cytokinesis 1 (PRO); RAD21 homolog (RAD21); Ras association (RalGDS/AF-6); domain family 1 (RASSF1); Stromal antigen 1 (STAG1); Synuclein-c, breast cancer-specific protein 1 (SNCG, BCSG1); Targeting protein for Xklp2 (TPX2); Transforming, acidic coiled-coil containing protein 3 (TACC3); Ubiquitin-conjugating enzyme E2C (UBE2C); Ubiquitin-conjugating enzyme E2I (UBE2I/UBC9); ZW10 interactor, (ZWINT); ZW10, kinetochore-associated homolog (ZW10); Zwilch, kinetochore-associated homolog (ZWILCH)
Ribonucleoprotein complexes	Argonaute family member, Agol, Ago2, Ago3, Ago4, GW1 82 (TNRC6A), TNRC6B, TNRC6C, HNRNPA2B1, HNRPAB, ILF2, NCL (Nucleolin), NPM1 (Nucleophosmin), RPL10A, RPL5, RPLP1, RPS12, RPS19, SNRPG, TROVE2, apolipoprotein, apolipoprotein A, apo A-I, apo A-II, apo A-IV, apo A-V, apolipoprotein B, apo B48, apo B100, apolipoprotein C, apo C-I, apo C-II, apo C-III, apo C-IV, apolipoprotein D (ApoD), apolipoprotein E (ApoE), apolipoprotein H (ApoH), apolipoprotein L, APOL1, APOL2, APOL3, APOL4, APOL5, APOL6, APOLD1

[00405] The instant disclosure provides various biomarkers that can be assessed in determining a biosignature for a given test sample, and which include assessment of polypeptides and/or nucleic acid biomarkers associated with various cancers, as well as the state of the cancer (e.g., metastatic v. non-metastatic).

[00406] In one example, a test sample can be assessed for a cancer by determining the presence or level of one or more biomarker including but not limited to CA-125, CA 19-9, and c-reactive protein. The cancer can be a cancer of the reproductive tract, e.g., an ovarian cancer. The one or more biomarker can further comprise one or more biomarkers, e.g., 1, 2, 3,4,5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more biomarkers, comprising one or more of CD95, FAP-1, miR-200 microRNAs, EGFR, EGFRvIII, apolipoprotein AI, apolipoprotein CIII, myoglobin, tenascin C, MSH6, claudin-3, claudin-4, caveolin-1, coagulation factor III, CD9, CD36, CD37, CD53, CD63, CD81, CD136, CD147, Hsp70, Hsp90, Rabl3, Desmocollin-1, EMP-2, CK7, CK20, GCDF15, CD82, Rab-5b, Annexin V, MFG-E8 and HLA-DR. MiR-200 microRNAs (i.e., the miR-200 microRNA family) comprises miR-200a, miR-200b, miR-200c, miR-141 and miR-429. Such assessment can include determining the presence or levels of proteins, nucleic acids, or both for each of the biomarkers disclosed herein.

[00407] CD95 (also called Fas, Fas antigen, Fas receptor, FasR, TNFRSF6, APT1 or APO-1) is a prototypical death receptor that regulates tissue homeostasis mainly in the immune system through the induction of apoptosis. During cancer progression, CD95 is frequently downregulated and the cells are rendered apoptosis resistant, thereby implicating loss of CD95 as part of a mechanism for tumour evasion. The tumorigenic activity of CD95 is mediated by a pathway involving JNK and Jun. FAP-1 (also referred to as Fas-associated phosphatase 1, protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase), PTPN13) is a member of the protein tyrosine phosphatase (PTP) family. FAP-1 has been reported to interact with, and dephosphorylate, CD95, thereby implicating a role in Fas mediated programmed cell death. MiR-200

PREOMICS EXHIBIT 1002
Page 1045 of 1861

family members can regulate CD95 and FAP-1. See Schickel et al. miR-200c regulates induction of apoptosis through CD95 by targeting FAP-1. Mol. Cell., 38, 908-915 (2010), which publication is incorporated by reference in its entirety herein.

[00408] Methods of the invention disclosed herein can use CD95 and/or FAP-1 characterization or profiling for microvesicle populations present in a biological sample to determine the presence of or predisposition to cancer, including without limitation any of the cancers disclosed herein. Methods of the invention comprising multiplexed analysis for multiple biomarkers use CD95 and/or FAP-1 biomarker characterization, along with other biomarkers disclosed herein, including but not limited to miR-200 microRNAs (e.g., miR-200c). In an embodiment, a biological test sample from an individual is assessed to determine the presence and level of CD95 and/or FAP-1 protein, or a presence or level of a CD95+ and/or FAP-1+ circulating microvesicle ("cMV") population, and the presence or levels are compared to a reference (e.g., samples from non-disease or normal, pre-treatment, or different treatment timepoints). This comparison is used to characterize the test sample. For example, comparison of the presence or levels of CD95 protein, FAP-1 protein, CD95+ cMVs and/or FAP-1+ cMVs in the test sample and reference are used to determine a disease phenotype or predict a response/non-response to treatment. In related embodiments, the cMV population is further assessed to determine a presence or level of miR-200 microRNAs, which are predetermined in a training set of reference samples to be indicative of disease or other prognostic, theranostic or diagnostic readout. Increased levels of FAP-1 in the test sample as compared to a non-cancer reference may indicate the presence of a cancer, or the presence of a more aggressive cancer. Decreased levels of CD95 or miR200 family members such as miR-200c as compared to a non-cancer reference may indicate the presence of a cancer, or the presence of a more aggressive cancer. The cMV population to be assessed can be isolated through immunoprecipitation, flow cytometry, or other isolation methodology disclosed herein or known in the art.

[00409] In a related aspect, the invention provides a method of characterizing a cancer comprising detecting a level of one or more biomarker, e.g., 1, 2, 3,4,5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 biomarkers, selected from the group consisting of A2ML1, BAX, C10orf47, Clorfl62, CSDA, EIFC3, ETFB, GABARAPL2, GUK1, GZMH, HIST1H3B, HLA-A, HSP90AA1, NRGN, PRDX5, PTMA, RABAC1, RABAGAP1L, RPL22, SAP18, SEPW1, SOX1, and a combination thereof. The one or more biomarker can comprise PTMA (prothymosin, alpha), a member of the pro/parathymosin family which is cleaved into Thymosin alpha-1 and has a role in immune modulation. Thymosin alpha-1 is approved in at least 35 countries for the treatment of Hepatitis B and C, and it is also approved for inclusion with vaccines to boost the immune response in the treatment of other diseases. In an embodiment, the biomarkers comprise mRNA. The mRNAs can be isolated from vesicles that have been isolated as described herein. In some embodiments, a total vesicle population in a sample is isolated, e.g., by filtration or centrifugation. The vesicles can also by isolated by affinity, e.g., using a binding agent to a general vesicle biomarker, a disease biomarker or a cell-specific biomarker.The levels of the biomarkers can be compared to a control such as a sample without cancer, wherein a change between the levels of the biomarkers versus the control is used to characterize the cancer. The cancer can be a prostate cancer.

[00410] In an embodiment, the cancer assessed by the invention comprises prostate cancer and microRNAs (miRs) are used to differentiate between metastatic versus non-metastatic prostate cancer. Prostate cancer staging is a process of categorizing the risk of cancer spread beyond the prostate. Such spread is related to the

PREOMICS EXHIBIT 1002
Page 1046 of 1861

probability of being cured with local therapies such as surgery or radiation. The information considered in such prognostic classification is based on clinical and pathological factors, including physical examination, imaging studies, blood tests and/or biopsy examination.

[00411] The most common scheme used to stage prostate cancer is promulgated by the American Joint Committee on Cancer, and is referred to as the TNM system. The TNM system evaluates the size of the tumor, the extent of involved lymph nodes, metastasis and also takes into account cancer grade. As with many other cancers, the cancers are often grouped by stage, e.g., stages I-IV). Generally, Stage I disease is cancer that is found incidentally in a small part of the sample when prostate tissue was removed for other reasons, such as benign prostatic hypertrophy, and the cells closely resemble normal cells and the gland feels normal to the examining finger. In Stage II more of the prostate is involved and a lump can be felt within the gland. In Stage III, the tumor has spread through the prostatic capsule and the lump can be felt on the surface of the gland. In Stage IV disease, the tumor has invaded nearby structures, or has spread to lymph nodes or other organs.

[00412] The Whitmore-Jewett stage is another staging scheme that is now used less often. The Gleason Grading System is based on cellular content and tissue architecture from biopsies, which provides an estimate of the destructive potential and ultimate prognosis of the disease.

[00413] The TNM tumor classification system can be used to describe the extent of cancer in a subject's body. T describes the size of the tumor and whether it has invaded nearby tissue, N describes regional lymph nodes that are involved, and M describes distant metastasis. TNM is maintained by the International Union Against Cancer (UICC) and is used by the American Joint Committee on Cancer (AJCC) and the International Federation of Gynecology and Obstetrics (FIGO). Those of skill in the art understand that not all tumors have TNM classifications such as, e.g., brain tumors. Generally, T (a,is,(0), 1-4) is measured as the size or direct extent of the primary tumor. N (0-3) refers to the degree of spread to regional lymph nodes: N Omeans that tumor cells are absent from regional lymph nodes, N1 means that tumor cells spread to the closest or small numbers of regional lymph nodes, N2 means that tumor cells spread to an extent between N1 and N3; N3 means that tumor cells spread to most distant or numerous regional lymph nodes. M (0/1) refers to the presence of metastasis: MX means that distant metastasis was not assessed; M0 means that no distant metastasis are present; M1 means that metastasis has occurred to distant organs (beyond regional lymph nodes). M1 can be further delineated as follows: Mia indicates that the cancer has spread to lymph nodes beyond the regional ones; Mlb indicates that the cancer has spread to bone; and Mlc indicates that the cancer has spread to other sites (regardless of bone involvement). Other parameters may also be assessed. G (1-4) refers to the grade of cancer cells (i.e., they are low grade if they appear similar to normal cells, and high grade if they appear poorly differentiated). R (0/1/2) refers to the completeness of an operation (i.e., resection-boundaries free of cancer cells or not). L (0/1) refers to invasion into lymphatic vessels. V (0/1) refers to invasion into vein. C (1-4) refers to a modifier of the certainty (quality) of V.

[00414] Prostate tumors are often assessed using the Gleason scoring system. The Gleason scoring system is based on microscopic tumor patterns assessed by a pathologist while interpreting a biopsy specimen. When prostate cancer is present in the biopsy, the Gleason score is based upon the degree of loss of the normal glandular tissue architecture (i.e. shape, size and differentiation of the glands). The classic Gleason scoring system has five basic tissue patterns that are technically referred to as tumor "grades." The microscopic determination of this loss of normal glandular structure caused by the cancer is represented by a grade, a number

PREOMICS EXHIBIT 1002
Page 1047 of 1861

ranging from 1 to 5, with 5 being the worst grade. Grade 1 is typically where the cancerous prostate closely resembles normal prostate tissue. The glands are small, well-formed, and closely packed. At Grade 2 the tissue still has well-formed glands, but they are larger and have more tissue between them, whereas at Grade 3 the tissue still has recognizable glands, but the cells are darker. At high magnification, some of these cells in a Grade 3 sample have left the glands and are beginning to invade the surrounding tissue. Grade 4 samples have tissue with few recognizable glands and many cells are invading the surrounding tissue. For Grade 5 samples, the tissue does not have recognizable glands, and are often sheets of cells throughout the surrounding tissue.

[00415] miRs that distinguish metastatic and non-metastatic prostate cancer can be overexpressed in metastatic samples versus non-metastatic. Alternately, miRs that distinguish metastatic and non-metastatic prostate cancer can be overexpressed in non-metastatic samples versus metastatic. Useful miRs for distinguishing metastatic prostate cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7 or 8, miRs selected from the group consisting of miR-495, miR-lOa, miR-30a, miR-570, miR-32, miR-885-3p, miR-564, and miR-134. In another embodiment, miRs for distinguishing metastatic prostate cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7, 8, 9, 10, 11, 12, 13 or 14, miRs selected from the group consisting of hsa-miR-375, hsa-miR-452, hsa-miR-200b, hsa-miR-146b-5p, hsa-miR-1296, hsa-miR-17*, hsa-miR-100, hsa-miR-574-3p, hsa-miR-20a*, hsa-miR-572, hsa-miR-1236, hsa-miR-181a, hsa-miR-937, and hsa-miR-23a*. In still another embodiment, useful miRs for distinguishing metastatic prostate cancer include, e.g., 1, 2, 3,4,5, 6, 7, 8 or 9, miRs selected from the group consisting of hsa-miR-200b, hsa-miR-375, hsa-miR-582-3p, hsa-miR-17*, hsa-miR-1296, hsa-miR-20a*, hsa-miR-100, hsa-miR-452, and hsa-miR-577. The miRs for distinguishing metastatic prostate cancer can be one or more, e.g., 1, 2, 3 or 4, miRs selected from the group consisting of miR-141, miR-375, miR-200b and miR-574-3p.

[00416] In an aspect, microRNAs (miRs) are used to differentiate between cancer and non-cancer samples. Vesicles derived from patient samples can be analyzed for miR payload contained within the vesicles. The sample can be a bodily fluid, including semen, urine, blood, serum or plasma. The sample can also comprise a tissue or biopsy sample. A number of different methodologies are available for detecting miRs. In some embodiments, arrays of miR panels are use to simultaneously query the expression of multiple miRs. The Exiqon mIRCURY LNA microRNA PCR system panel (Exiqon, Inc., Woburn, MA) can be used for such purposes. miRs that distinguish cancer can be overexpressed in cancer versus control samples. Alternately, miRs that distinguish cancer can be overexpressed in cancer samples versus controls. Useful miRs for distinguishing cancer from non-cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7, 8, 9, 10, 11, 12 or 13, miRs selected from the group consisting of hsa-miR-574-3p, hsa-miR-33 1-3p, hsa-miR-326, hsa-miR-1 81a-2*, hsa-miR-130b, hsa-miR-301a, hsa-miR-141, hsa-miR-432, hsa-miR-107, hsa-miR-628-5p, hsa-miR-625*, hsa-miR-497, and hsa-miR-484. In another embodiment, useful miRs for distinguishing cancer from non-cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7, 8, 9 or 10, miRs selected from the group consisting of hsa-miR-574-3p, hsa-miR-141, hsa-miR-33 1-3p, hsa-miR-432, hsa-miR-326, hsa-miR-21 10, hsa-miR-107, hsa-miR-130b, hsa-miR-301a, and hsa-miR-625*. In still another embodiment, the useful miRs for distinguishing cancer from non-cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7, 8 or 9, miRs selected from the group consisting of hsa-miR-107, hsa-miR-326, hsa-miR-432, hsa-miR-574-3p, hsa-miR-625*, hsa-miR-21 10, hsa-miR-301a, hsa-miR-141 or hsa-miR-373*. The cancer can comprise those cancers listed above. In an exemplary embodiment, the cancer is a prostate cancer and the microRNAs (miRs) are used to differentiate between prostate cancer and non-cancer samples.

PREOMICS EXHIBIT 1002
Page 1048 of 1861

[00417] The method contemplates assessing combinations of circulating biomarkers. For example, multiple markers from antibody arrays and miR analysis can be used to distinguish prostate cancer from normal, BPH and PCa, or metastatic versus non-metastatic disease. In this manner, improved sensitivity, specificity, and/or accuracy can be obtained. In some embodiments, the levels of one or more, e.g., 1, 2, 3,4,5 or 6, miRs selected from the group consisting of hsa-miR-432, hsa-miR-143, hsa-miR-424, hsa-miR-204, hsa-miR-581f and hsa-miR-45 1 are detected in a patient sample to assess the presence of prostate cancer. Any of these miRs can be elevated in patients with PCa but having serum PSA < 4.0 ng/ml. In an embodiment, the invention provides a method of assessing a prostate cancer, comprising determining a level of one or more, e.g., 1, 2, 3,4,5 or 6, miRs selected from the group consisting of hsa-miR-432, hsa-miR-143, hsa-miR-424, hsa-miR-204, hsa-miR-581f and hsa-miR-45 1 in a sample from a subject. The sample can be a bodily fluid, e.g., blood, plasma or serum. The miRs can be isolated in vesicles isolated from the sample. The subject can have a PSA level less than some threshold, such as 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, or 6.0 ng/ml in a blood sample. Higher levels of the miRs than in a reference sample can indicate the presence of PCa in the sample. In some embodiments, the reference comprises a level of the one or more miRs in control samples from subjects without PCa. In some embodiments, the reference comprises a level of the one or more miRs in control samples from subject with PCa and PSA levels ≥ some threshold, such as 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, or 6.0 ng/ml. The threshold can be 4.0 ng/ml.

[00418] In some embodiments of the invention, vesicles in patient samples are assessed to provide a diagnostic, prognostic or theranostic readout. Vesicle analysis of patient samples includes the detection of vesicle surface biomarkers, e.g., surface antigens, and/or vesicle payload, e.g., mRNAs and microRNAs, as described herein. Methods for analysis of vesicles are presented in PCT Patent Application PCT/US09/06095, entitled "METHODS AND SYSTEMS OF USING EXOSOMES FOR DETERMINING PHENOTYPES" and filed November 12, 2009; U.S. Provisional Patent Application 61/362,674, entitled "METHODS AND SYSTEMS OF USING VESICLES FOR DETERMINING PHENOTYPES" and filed July 7, 2010; and U.S. Provisional Patent Application 61/393,823, entitled "DETECTION OF GI CANCERS" and filed October 15, 2010, which applications are incorporated by reference herein in their entirety.

[00419] In one aspect, the invention includes a method of identifying a bio-signature of one or more vesicles in a biological sample from said subject, wherein the bio-signature comprises analysis of vesicle surface antigens and vesicle payload. The surface antigens can comprise surface proteins and the vesicle payload can comprise microRNA. For example, vesicles can be captured using binding agents that recognize vesicle surface antigens, and the microRNA inside these captured vesicles can be assessed. Accordingly, the bio-signature may comprise the surface antigens used for capture as well as the microRNA inside the vesicles. The bio-signature can be used for diagnostic, prognostic or theranostic purposes. For example, the bio-signature can be a signature that identifies cancer, identifies aggressive or metastatic cancer, or identifies a cancer that is likely to respond to a candidate therapeutic agent.

[00420] As an illustrative example, consider a method of capturing vesicles in a sample using an antibody to B7H3 and then assessing the levels of miR-141 within the captured vesicles. In this example, the bio-signature comprises the level of miR-141 within exosomes displaying B7H3 on their surface. Depending on the levels of B7H3+ vesicles in the sample as well as the levels of miR-141 within the sample, the bio-signature may indicate

PREOMICS EXHIBIT 1002
Page 1049 of 1861

that the sample comprises a cancer, comprises an aggressive cancer, is likely to respond to a certain treatment or chemotherapeutic agent, etc.

[00421] In one embodiment, the method of assessing cancer in a subject comprises: identifying a bio-signature of one or more vesicles in a biological sample from said subject, comprising: determining a level of one or more general vesicles protein biomarkers; determining a level of one or more cell-specific protein biomarkers; determining a level of one or more disease-specific protein biomarkers; and determining the level of one or more microRNA biomarkers in the vesicles, wherein said characterizing comprises comparing said levels of biomarkers in said sample to a reference to determine whether said subject may be predisposed to or afflicted with cancer. The protein biomarkers can be detected in a multiplex fashion in a single assay. The microRNA biomarkers can also be detected in a multiplex fashion in a single assay. In some cases, the cell-specific and disease-specific biomarker may overlap, e.g., one biomarker may serve to identify a cancer from a particular cellular origin. The biological sample can be a bodily fluid, such as blood, serum or plasma.

[00422] In an example, the method of the invention comprises a diagnostic test for prostate cancer comprising isolating vesicles from a blood sample from a patient to detect vesicles indicative of the presence or absence of prostate cancer. The blood can be serum or plasma. The vesicles are isolated by capture with "capture antibodies" that recognize specific vesicle surface antigens. The surface antigens for the prostate cancer diagnostic assay include the tetraspanins CD9, CD63 and CD81, which are generally present on vesicles in the blood and therefore act as general vesicle biomarkers, the prostate specific biomarkers PSMA and PCSA, and the cancer specific biomarker B7H3. In some cases, EpCam is used as a cancer specific biomarker as well or instead of B7H3. The capture antibodies can be tethered to a substrate. In an embodiment, the substrate comprises fluorescently labeled beads, wherein the beads are differentially labeled for each capture antibody. As desired, the payload of the detected vesicles can be assessed in order to characterize the cancer.

[00423] As described above, the biomarkers of the invention can be assessed to identify a biosignature. In an aspect, the invention provides a method comprising: determining a presence or level of one or more biomarker in a biological sample, wherein the one or more biomarker comprises one or more biomarker selected from **Table 5**; and identifying a biosignature comprising the presence or level of the one or more biomarker. In some embodiments, the method further comprises comparing the biosignature to a reference biosignature, wherein the comparison is used to characterize a cancer, including the cancers disclosed herein or known in the art. The reference biosignature can be from a subject without the cancer. The reference biosignature can also be from the subject, e.g., from normal adjacent tissue or from a sample taken at another point in time. Various ways of characterizing a cancer are disclosed herein. For example, characterizing the cancer may comprise identifying the presence or risk of the cancer in a subject, or identifying the cancer in a subject as metastatic or aggressive. The comparing step comprises determining whether the biosignature is altered relative to the reference biosignature, thereby providing a prognostic, diagnostic or theranostic characterization for the cancer. The biological sample comprises a bodily fluid, including without limitation the bodily fluids disclosed herein. For example, the bodily fluid may comprise urine, blood or a blood derivative.

[00424] The one or more biomarker can be one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more, selected from the group consisting of miR-22, let7a, miR-141, miR-182, miR-663, miR-155, mirR-125a-5p, miR-548a-5p, miR-628-5p, miR-517*, miR-450a, miR-920, hsa-miR-619, miR-1913, miR-224*, miR-502-5p, miR-888, miR-376a, miR-542-5p, miR-30b*, miR-1179, and a combination thereof. In an embodiment, the one

PREOMICS EXHIBIT 1002
Page 1050 of 1861

or more biomarker is selected from the group consisting of miR-22, let7a, miR-141, miR-920, miR-450a, and a combination thereof. The one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more, may be a messenger RNA (mRNA) selected from the group consisting of the genes in any of **Tables 20-24** herein, and a combination thereof. For example, the one or more biomarker may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more messenger RNA (mRNA) selected from the group consisting of A2ML1, BAX, C10orf47, Clorfl62, CSDA, EIFC3, ETFB, GABARAPL2, GUK1, GZMH, HIST1H3B, HLA-A, HSP90AA1, NRGN, PRDX5, PTMA, RABAC1, RABAGAP1L, RPL22, SAP 18, SEPW1, SOX1, and a combination thereof. The one or more biomarker may comprise 1, 2, 3, 4, 5, or 6 messenger RNA (mRNA) selected from the group consisting of A2ML1, GABARAPL2, PTMA, RABAC1, SOX1, EFTB, and a combination thereof. The one or more biomarker may be isolated as payload of a population of microvesicles. The population can be a total population of microvesicles from the sample or a specific population, such as a PCSA+ population. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer.

[00425] In an embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of CA-125, CA 19-9, c-reactive protein, CD95, FAP-1, EGFR, EGFRvIII, apolipoprotein AI, apolipoprotein CIII, myoglobin, tenascin C, MSH6, claudin-3, claudin-4, caveolin-1, coagulation factor III, CD9, CD36, CD37, CD53, CD63, CD81, CD136, CD147, Hsp70, Hsp90, Rabl3, Desmocollin-1, EMP-2, CK7, CK20, GCDF15, CD82, Rab-5b, Annexin V, MFG-E8, HLA-DR, a miR200 microRNA, miR-200c, and a combination thereof. The one or more biomarker may comprise 1, 2, 3, 4 or 5 biomarker selected from the group consisting of CA-125, CA 19-9, c-reactive protein, CD95, FAP-1, and a combination thereof. The one or more biomarker may be isolated directly from sample, or as surface antigens or payload of a population of microvesicles. In an embodiment, the method is used to assess an ovarian cancer. For example, the method can be used to distinguish a sample comprising ovarian cancer from a sample without ovarian cancer. Altenarately, the method can be used to distinguish amongst ovarian cancer having different stage or prognosis.

[00426] In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of hsa-miR-574-3p, hsa-miR-141, hsa-miR-432, hsa-miR-326, hsa-miR-21 10, hsa-miR-1 81a-2*, hsa-miR-107, hsa-miR-301a, hsa-miR-484, hsa-miR-625*, and a combination thereof. The method can be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. In still another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of hsa-miR-582-3p, hsa-miR-20a*, hsa-miR-375, hsa-miR-200b, hsa-miR-379, hsa-miR-572, hsa-miR-5 13a-5p, hsa-miR-577, hsa-miR-23a*, hsa-miR-1236, hsa-miR-609, hsa-miR-17*, hsa-miR-130b, hsa-miR-619, hsa-miR-624*, hsa-miR-198, and a combination thereof. For example, the method can be used to distinguish a sample comprising metastatic prostate cancer from a sample with non-metastatic prostate cancer. The one or more biomarker may be isolated as payload of a population of microvesicles.

[00427] The one or more biomarker may be miR-497. The method can be used to assess a lung cancer. For example, the method can be used to distinguish a lung cancer sample from a non-cancer sample. The one or more biomarker may be isolated as payload of a population of microvesicles.

PREOMICS EXHIBIT 1002
Page 1051 of 1861

[00428] The one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, may comprise a messenger RNA (mRNA) selected from the group consisting of AQP2, BMP5, C16orf86, CXCL13, DST, ERCCl, GNAOl, KLHL5, MAP4K1, NELL2, PENK, PGF, POU3F 1, PRSS21, SCMLl, SEMGl, SMARCD3, SNAI2, TAF 1C, TNNT3, and a combination thereof. The mRNA may be isolated from microvesicles. The method can be used to characterize a prostate cancer, such as distinguish a prostate cancer sample from a normal sample without cancer. In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, comprises a messenger RNA (mRNA) selected from the group consisting of ADRB2, ARG2, C22orG2, CYorfl4, EIF1AY, FEV, KLK2, KLK4, LRRC26, MAOA, NLGN4Y, PNPLA7, PVRL3, SIM2, SLC30A4, SLC45A3, STX19, TRIM36, TRPM8, and a combination thereof. The mRNA may be isolated from microvesicles. The method can be used to characterize a prostate cancer, such as distinguish a prostate cancer sample from a sample having another cancer, e.g., a breast cancer. In still another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, comprises a messenger RNA (mRNA) selected from the group consisting of ADRB2, BAIAP2L2, C19orG3, CDX1, CEACAM6, EEF1A2, ERN2, FAM1 10B, FOXA2, KLK2, KLK4, LOC389816, LRRC26, MIPOLl, SLC45A3, SPDEF, TRIM3 1, TRIM36, ZNF613, and a combination thereof. The mRNA may be isolated from microvesicles. The method can be used to characterize a prostate cancer, such as distinguish a prostate cancer sample from a sample having another cancer, e.g., a colorectal cancer. In yet another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, comprises a messenger RNA (mRNA) selected from the group consisting of ASTN2, CAB39L, CRIPl, FAM1 10B, FEV, GSTP1, KLK2, KLK4, LOC389816, LRRC26, MUC1, PNPLA7, SIM2, SLC45A3, SPDEF, TRIM36, TRPV6, ZNF613, and a combination thereof. The mRNA may be isolated from microvesicles. The method can be used to characterize a prostate cancer, such as distinguish a prostate cancer sample from a sample having another cancer, e.g., a lung cancer. The one or more biomarker can also be a microRNA that regulates one or more of the mRNAs used to characterize a prostate cancer. For example, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, may comprise a microRNA selected from the group consisting of miRs-26a+b, miR-15, miR-16, miR-195, miR-497, miR-424, miR-206, miR-342-5p, miR-1 86, miR-1271, miR-600, miR-216b, miR-519 family, miR-203, and a combination thereof. The microRNA can be assessed as payload of a microvesicle population.

[00429] In still another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or more biomarkers, is selected from the group consisting of A33, ABL2, ADAM 10, AFP, ALA, ALFX, ALPL, ApoJ/CLU, ASCA, ASPH(A-IO), ASPH(DOIP), AURKB, B7H3, B7H3, B7H4, BCNP, BDNF, CA125(MUC16), CA-19-9, C-Bir, CDIO, CD15 1, CD24, CD41, CD44, CD46, CD59(MEM-43), CD63, CD63, CD66eCEA, CD81, CD81, CD9, CD9, CDA, CDADC1, CRMP-2, CRP, CXCL12, CXCR3, CYFRA21-1, DDX-1, DLL4, DLL4, EGFR, Epcam, EphA2, ErbB2, ERG, EZH2, FASL, FLNA, FRT, GAL3, GATA2, GM-CSF, Gro-alpha, HAP, HER3(ErbB3), HSP70, HSPB1, hVEGFR2, iC3b, IL-1B, IL6R, IL6Unc, IL7Ralpha/CD127, IL8, INSIG-2, Integrin, KLK2, LAMN, Mammoglobin, M-CSF, MFG-E8, MIF, MISRII, MMP7, MMP9, MUC1, Mucl, MUC17, MUC2, Ncam, NDUFB7, NGAL, NK-2R(C-21), NT5E (CD73), p53, PBP, PCSA, PCSA, PDGFRB, PIM1, PRL, PSA, PSA, PSMA, PSMA, RAGE, RANK, ReglV, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2(C-15), SPARC, SPC, SPDEF, SPP1, STEAP, STEAP4, TFF3, TGM2, TIMP-1, TMEM21 1, Trail-R2, Trail-R4, TrKB(poly), Trop2, TsglOl, TWEAK, UNC93A, VEGFA, wnt-5a(C-16), and a combination thereof. The one or more biomarker may be detected directly in a sample, or as surface

PREOMICS EXHIBIT 1002
Page 1052 of 1861

antigens or payload of a population of microvesicles. In an embodiment, a binding agent to the one or more biomarker is used to capture a microvesicle population. The captured microvesicle population can be detected using another binding agent, e.g., a labeled binding agent to a general vesicle marker such as one or more protein in **Table 3,** or a cell-of-origin or or cancer-specific biomarker. In an embodiment, the antigen used for detection comprises one or more of CD9, CD63, CD81, PCSA, MUC2, and MFG-E8. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. Altenarately, the method is used to distinguish amongst prostate cancers having different stage or prognosis.

[00430] In a related embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or more biomarkers, is selected from the group consisting of A33, ADAM 10, AMACR, ASPH (A-10), AURKB, B7H3, CA125, CA-19-9, C-Bir, CD24, CD3, CD41, CD63, CD66e CEA, CD81, CD9, CDADC1, CSA, CXCL12, DCRN, EGFR, EphA2, ERG, FLNA, FRT, GAL3, GM-CSF, Gro-alpha, HER 3 (ErbB3), hVEGFR2, IL6 Unc, Integrin, Mammaglobin, MFG-E8, MMP9, MUC1, MUC17, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, PBP, PCSA, PIMl, PRL, PSA, PSIPl/LEDGF, PSMA, RANK, S100-A4, seprase/FAP, SIM2 (C-15), SPDEF, SSX2, STEAP, TGM2, TIMP-1, Trail-R4, Tsg 101, TWEAK, UNC93A, VCAN, XAGE-1, and a combination thereof. The one or more biomarker may be detected directly in a sample, or as surface antigens or payload of a population of microvesicles. In an embodiment, a binding agent to the one or more biomarker is used to capture a microvesicle population. The captured microvesicle population can be detected using another binding agent, e.g., a labeled binding agent to a general vesicle marker such as one or more protein in **Table 3,** or a cell-of-origin or or cancer-specific biomarker. In an embodiment, the antigen used for detection comprises one or more of EpCAM, CD81, PCSA, MUC2 and MFG-E8. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. Altenarately, the method is used to distinguish amongst prostate cancers having different stage or prognosis.

[00431] In another related embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or more biomarkers, is selected from the group consisting of A33, ADAM 10, ALIX, AMACR, ASCA, ASPH (A-10), AURKB, B7H3, BCNP, CA125, CA-19-9, C-Bir (Flagellin), CD24, CD3, CD41, CD63, CD66e CEA, CD81, CD9, CDADC1, CRP, CSA, CXCL12, CYFRA21-1, DCRN, EGFR, EpCAM, EphA2, ERG, FLNA, GAL3, GATA2, GM-CSF, Gro alpha, HER3 (ErbB3), HSP70, hVEGFR2, iC3b, IL-1B, IL6 Unc, IL8, Integrin, KLK2, Mammaglobin, MFG-E8, MMP7, MMP9, MS4A1, MUC1, MUC17, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, p53, PBP, PCSA, PIMl, PRL, PSA, PSMA, RANK, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2 (C-15), SPC, SPDEF, SSX2, SSX4, STEAP, TGM2, TIMP-1, TRAIL R2, Trail-R4, Tsg 101, TWEAK, VCAN, VEGF A, XAGE, and a combination thereof. The one or more biomarker may be detected directly in a sample, or as surface antigens or payload of a population of microvesicles. In an embodiment, a binding agent to the one or more biomarker is used to capture a microvesicle population. The captured microvesicle population can be detected using another binding agent, e.g., a labeled binding agent to a general vesicle marker such as one or more protein in **Table 3,** or a cell-of-origin or or cancer-specific biomarker. In an embodiment, the antigen used for detection comprises one or more of EpCAM, CD81, PCSA, MUC2 and MFG-E8. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish

PREOMICS EXHIBIT 1002
Page 1053 of 1861

a sample comprising prostate cancer from a sample without prostate cancer. Altenarately, the method is used to distinguish amongst prostate cancers having different stage or prognosis.

[00432] In still another related embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 biomarkers, is selected from the group consisting of ADAM- 10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, SSX4, and a combination thereof. The one or more biomarker may be detected directly in a sample, or as surface antigens or payload of a population of microvesicles. In an embodiment, a binding agent to the one or more biomarker is used to capture a microvesicle population. The captured microvesicle population can be detected using another binding agent, e.g., a labeled binding agent to a general vesicle marker such as one or more protein in **Table 3,** or a cell-of-origin or or cancer-specific biomarker. In an embodiment, the antigen used for detection comprises one or more of EpCAM, KLK2, PBP, SPDEF, SSX2, SSX4. In a non-limiting example, consider that the detector binding agent is a binding agent to EpCam, e.g., an antibody or aptamer to EpCam, wherein the antibody or aptamer is optionally labeled to facilitate detection thereof. In such case, the one or more biomarker comprises one or more pair of biomarkers selected from the group consisting of EpCam - ADAM- 10, EpCam - BCNP, EpCam - CD9, EpCam - EGFR, EpCam - EpCam, EpCam - IL1B, EpCam - KLK2, EpCam - MMP7, EpCam - p53, EpCam - PBP, EpCam - PCSA, EpCam - SERPINB3, EpCam - SPDEF, EpCam - SSX2, EpCam - SSX4, and a combination thereof. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. Altenarately, the method is used to distinguish amongst prostate cancers having different stage or prognosis.

[00433] In one embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of miR-148a, miR-329, miR-9, miR-378*, miR-25, miR-614, miR-5 18c*, miR-378, miR-765, let-7f-2*, miR-574-3p, miR-497, miR-32, miR-379, miR-520g, miR-542-5p, miR-342-3p, miR-1206, miR-663, miR-222, and a combination thereof. In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of hsa-miR-877*, hsa-miR-593, hsa-miR-595, hsa-miR-300, hsa-miR-324-5p, hsa-miR-548a-5p, hsa-miR-329, hsa-miR-550, hsa-miR-886-5p, hsa-miR-603, hsa-miR-490-3p, hsa-miR-938, hsa-miR-149, hsa-miR-150, hsa-miR-1296, hsa-miR-384, hsa-miR-487a, hsa-miRPlus-C1089, hsa-miR-485-3p, hsa-miR-525-5p, and a combination thereof. The method can be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. In still another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of miR-588, miR-1258, miR-16-2*, miR-938, miR-526b, miR-92b*, let-7d, miR-378*, miR-124, miR-376c, miR-26b, miR- 1204, miR-574-3p, miR-195, miR-499-3p, miR-21 10, miR-888, and a combination thereof. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample with inflammatory prostate disease. The one or more biomarker may be isolated as payload of a population of microvesicles.

[00434] In one embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of let-7d, miR-148a, miR-195, miR-25, miR-26b, miR-329, miR-376c, miR-574-3p, miR-888, miR-9, miR1204, miR-16-2*, miR-497, miR-588, miR-614, miR-765, miR92b*, miR-938, let-7f-2*, miR-300, miR-523, miR-525-5p, miR-1 182, miR-1244, miR-520d-3p, miR-379, let-7b, miR-125a-3p, miR-1296, miR-134, miR-149, miR-150, miR-1 87, miR-32, miR-324-3p, miR-324-5p, miR-342-3p, miR-378,

PREOMICS EXHIBIT 1002
Page 1054 of 1861

miR-378*, miR-384, miR-451, miR-455-3p, miR-485-3p, miR-487a, miR-490-3p, miR-502-5p, miR-548a-5p, miR-550, miR-562, miR-593, miR-593*, miR-595, miR-602, miR-603, miR-654-5p, miR-877*, miR-886-5p, miR-125a-5p, miR-140-3p, miR-192, miR-196a, miR-2110, miR-212, miR-222, miR-224*, miR-30b*, miR-499-3p, miR-505*, and a combination thereof. In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of hsa-miR-451, hsa-miR-223, hsa-miR-593*, hsa-miR-1974, hsa-miR-486-5p, hsa-miR-19b, hsa-miR-320b, hsa-miR-92a, hsa-miR-21, hsa-miR-675*, hsa-miR-16, hsa-miR-876-5p, hsa-miR-144, hsa-miR-126, hsa-miR-137, hsa-miR-1913, hsa-miR-29b-1*, hsa-miR-15a, hsa-miR-93, hsa-miR-1266, and a combination thereof. The method can be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. The one or more biomarker may be isolated as payload of a population of microvesicles. The population can comprise PCSA+ microvesicles. In an embodiment, the population consists of PCSA+ microvesicles. In one embodiment, a population of PCSA+ vesicles is isolated and microRNA within the isolated vesicles are assessed using methods as described herein or known in the art. Elevated levels of miR-1974 in a test sample as compared to a control sample (e.g., non-cancer sample) are indicative of a prostate cancer in the test sample. Similarly, decreased levels of miR-320b in a test sample as compared to a control sample (e.g., non-cancer sample) can indicate the presence of a prostate cancer in the test sample.

[00435] The one or more biomarker can comprise EpCAM and MMP7. The biomarkers may be isolated from microvesicles. In an embodiment, EpCAM+ / MMP7+ microvesicles are detected in a sample, such as blood or a blood derivative. In a non-limiting example, the EpCAM+ / MMP7+ microvesicles are identified by EpCAM and MMP7 binding agents using methods as described herein, e.g., using flow cytometry. As described, vesicles in a biological sample can be identified by flow sorting using general vesicle markers, e.g., the marker in **Table 3** such as tetraspanins including CD9, CD63 and/or CD81. The levels of the EpCAM+ / MMP7+ microvesicles can be used to characterize a cancer, such as distinguish a cancer sample from a normal sample without cancer. In one embodiment, lower levels of MMP7 in EpCAM+ vesicles as compared to a non-cancer control sample indicate the presense of cancer. As EpCAM and MMP7 comprise cancer markers, one of skill will appreciate that the method can be used to assess various cancers in a sample. In an embodiment, the cancer comprises prostate cancer.

[00436] In another embodiment, the one or more biomarker comprises a transcription factor. The transcription factor can be one or more, e.g., 2, 3, 4, 5, 6, 7, 8, 9 or 10 of c-Myc, AEBP1, HNF4a, STAT3, EZH2, p53, MACC1, SPDEF, RUNX2 and YB-1. In another embodiment, the one or more biomarker may also comprise a kinase. The kinase can be one or more of AURKA and AURKB. The method can be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. The one or more biomarker may be isolated as payload of a population of microvesicles. In an embodiment, elevated levels of the transcription factors and/or kinases in the microvesicle population as compared to normal controls indicate the presence of a cancer. As these are cancer-related transcription factors, one of skill will appreciate that any appropriate cancer can be assessed using the method. In an embodiment, the cancer comprises a prostate cancer or a breast cancer.

[00437] The one or more biomarker can comprise PCSA, Muc2 and AdamlO. The biomarkers may be isolated from microvesicles. In an embodiment, PCSA+ / Muc2+ / Adaml0+ microvesicles are detected in a sample, such as blood or a blood derivative. In a non-limiting example, the PCSA+ / Muc2+ / Adaml0+ microvesicles

PREOMICS EXHIBIT 1002
Page 1055 of 1861

are identified by PCSA, Muc2 and AdamlO binding agents using methods as described herein, e.g., using flow cytometry. As described, vesicles in a biological sample can be identified by flow sorting using general vesicle markers, e.g., the marker in **Table 3** such as tetraspanins including CD9, CD63 and/or CD81 . The levels of the PCSA+ / Muc2+ / Adaml0+ microvesicles can be used to characterize a cancer, such as distinguish a cancer sample from a normal sample without cancer. In one embodiment, elevated levels of PCSA+ / Muc2+ / Adaml0+ vesicles as compared to a non-cancer control sample indicate the presense of cancer. In an embodiment, the cancer comprises prostate cancer.

[00438] In one embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting Alkaline Phosphatase (AP), CD63, MyoDl, Neuron Specific Enolase, MAPIB, CNPase, Prohibitin, CD45RO, Heat Shock Protein 27, Collagen II, Laminin Bl/bl, Gail, CDw75, bcl-XL, Laminin-s, Ferritin, CD21, ADP-ribosylation Factor (ARF-6). In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of CD56/NCAM-1, Heat Shock Protein 27/hsp27, CD45RO, MAPIB, MyoDl, CD45/T200/LCA, CD3zeta, Laminin-s, bcl-XL, Radl8, Gail, Thymidylate Synthase, Alkaline Phosphatase (AP), CD63, MMP-16 / MT3-MMP, Cyclin C, Neuron Specific Enolase, SIRP al, Laminin Bl/bl, Amyloid Beta (APP), SODD (Silencer of Death Domain), CDC37, Gab-1, E2F-2, CD6, Mast Cell Chymase, Gamma Glutamylcysteine Synthetase (GCS), and a combination thereof. The one or more biomarker can comprise protein. The one or more biomarker may be isolated as payload of a population of microvesicles. The method can be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a control sample without prostate cancer. The control sample can be a sample from a non-diseased state, a non-malignant prostate condition, or it can be a sample indicative of another type of cancer or related disorder, such as a breast cancer, brain cancer, lung cancer, colorectal cancer or colorectal adenoma. In an embodiment, elevated levels of Alkaline Phosphatase (AP) as compared to the control indicate the presence of prostate cancer. Similarly, elevated levels of CD56 (NCAM) as compared to the control can indicate the presence of prostate cancer. In an embodiment, elevated levels of CD-3 zeta as compared to the control indicate the presence of prostate cancer. In anther embodiment, elevated levels of Map1b as compared to the control can indicate the presence of prostate cancer. Conversely, elevated levels of 14.3.3 and/or filamin may indicate a colorectal cancer and not prostate cancer or other cancers or prostate disorders. Similarly, elevated levels of thrombospondin may indicate a colorectal or lung cancer and not prostate cancer or other cancers or prostate disorders.

[00439] In one embodiment, the one or more biomarker comprises MMP7. The one or more biomarker can comprise protein. The one or more biomarker may be a surface antigen or payload of a population of microvesicles. The method can be used to assess a cancer. One of skill will appreciate that any appropriate cancer can be assessed using the method as MMP7 is a known cancer marker. In an embodiment, the cancer comprises a prostate cancer.

[00440] In an aspect, the invention provides a method of identifying a biosignature by assessing biomarker complexes. In an aspect, the method comprises isolating one or more nucleic acid-protein complex from a biological sample; determining a presence or level of one or more nucleic acid biomarker with the one or more nucleic acid-protein complex; and identifying a biosignature comprising the presence or level of the one or more nucleic acid biomarker. In some embodiments, the biosignature may also comprise the presence or level of one

PREOMICS EXHIBIT 1002
Page 1056 of 1861

or more protein or other component of the complex. The nucleic acid-protein complex may be isolated from the biological sample using methodology disclosed herein or known in the art. For example, the complex may be isolated by affinity selection such as by immunoprecipitation, column chromatography or flow cytometry, using a binding agent to a component of the complex. Binding agents can be as described herein, e.g., an antibody or aptamer to a protein component of the complex. In some embodiments, the method further comprises comparing the biosignature to a reference biosignature, wherein the comparison is used to characterize a cancer, including the cancers disclosed herein or known in the art. The reference biosignature can be from a subject without the cancer. The reference biosignature can also be from the subject, e.g., from normal adjacent tissue or from a sample taken at another point in time. Various ways of characterizing a cancer are disclosed herein. For example, characterizing the cancer may comprise identifying the presence or risk of the cancer in a subject, or identifying the cancer in a subject as metastatic or aggressive. The comparing step comprises determining whether the biosignature is altered relative to the reference biosignature, thereby providing a prognostic, diagnostic or theranostic characterization for the cancer. The biological sample comprises a bodily fluid, including without limitation the bodily fluids disclosed herein. For example, the bodily fluid may comprise urine, blood or a blood derivative.

[00441] In an embodiment, the nucleic acid-protein complex comprises one or more protein, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more proteins, selected from the group consisting of one or more Argonaute family member, Agol, Ago2, Ago3, Ago4, GW1 82 (TNRC6A), TNRC6B, TNRC6C, HNRNPA2B1, HNRPAB, ILF2, NCL (Nucleolin), NPM1 (Nucleophosmin), RPL10A, RPL5, RPLP1, RPS12, RPS19, SNRPG, TROVE2, apolipoprotein, apolipoprotein A, apo A-I, apo A-II, apo A-IV, apo A-V, apolipoprotein B, apo B48, apo B100, apolipoprotein C, apo C-I, apo C-II, apo C-III, apo C-IV, apolipoprotein D (ApoD), apolipoprotein E (ApoE), apolipoprotein H (ApoH), apolipoprotein L, APOL1, APOL2, APOL3, APOL4, APOL5, APOL6, APOLD1, and a combination thereof. For example, the nucleic acid-protein complex may comprise one or more protein selected from the group consisting of one or more Argonaute family member, Agol, Ago2, Ago3, Ago4, GW1 82 (TNRC6A), and a combination thereof. The nucleic acid-protein complex comprises one or more protein selected from the group consisting of Ago2, Apolipoprotein I, GW1 82 (TNRC6A), and a combination thereof.

[00442] In embodiments, the one or more nucleic acid in the nucleic acid-protein complex comprises one or more microRNA. For example, the one or more microRNA, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50 or more microRNA, can be a microRNA in **Table 5.** The one or more microRNA may comprise one or more microRNA, e.g., 1, 2, 3, 4, 5 or 6 microRNA, selected from the group consisting of miR-22, miR-16, miR-148a, miR-92a, miR-45 1, let7a, and a combination thereof. The one or more microRNA may be assessed in order to characterize, e.g., diagnose, prognose or theranose, a cancer including without limitation a prostate cancer.

[00443] In an embodiment, the nucleic acid-protein complex comprises one or more protein selected from the group consisting of Ago2, Apolipoprotein I, GW1 82 (TNRC6A), and a combination thereof; and the one or more microRNA comprises one or more microRNA selected from the group consisting of miR-16 and miR-92a, and a combination thereof. The one or more microRNA may be assessed in order to characterize a prostate cancer.

[00444] The invention further provides a method of determining a biosignature comprising detecting nucleic acids in microvesicle population of interest. The vesicle population can be a whole population in a biological

PREOMICS EXHIBIT 1002
Page 1057 of 1861

sample, or a subpopulation such as a subpopulation having certain surface antigens. The method comprises detecting one or more protein biomarker in a microvesicle population from a biological sample; determining a presence or level of one or more one or more nucleic acid biomarker associated with the detected microvesicle population; and identifying a biosignature comprising the presence or level of the one or more nucleic acid. Techniques for detecting microvesicle populations, detecting proteins, and assessing nucleic acids can be disclosed herein or as known in the art. For example, the microvesicles can be isolated by affinity selection against the one or more protein, and nucleic acid can be isolated from the selected microvesicles. The level of the one or more one or more nucleic acid biomarker can be normalized to the level of the one or more protein biomarker or to the level of the microvesicle population. In some embodiments, the method further comprises comparing the biosignature to a reference biosignature, wherein the comparison is used to characterize a cancer, including the cancers disclosed herein or known in the art. The reference biosignature can be from a subject without the cancer. The reference biosignature can also be from the subject, e.g., from normal adjacent tissue or from a sample taken at another point in time. Various ways of characterizing a cancer are disclosed herein. For example, characterizing the cancer may comprise identifying the presence or risk of the cancer in a subject, or identifying the cancer in a subject as metastatic or aggressive. The comparing step comprises determining whether the biosignature is altered relative to the reference biosignature, thereby providing a prognostic, diagnostic or theranostic characterization for the cancer. The biological sample comprises a bodily fluid, including without limitation the bodily fluids disclosed herein. For example, the bodily fluid may comprise urine, blood or a blood derivative.

[00445] The proteins used for detecting one or more protein biomarker in a microvesicle population may comprise one or more biomarker disclosed herein, such as in **Tables 3-5** or **9-11.** For example, the one or more protein can be selected from the group consisting of PCSA, Ago2, CD9 and a combination thereof. For example, the one or more protein can be PCSA, Ago2, CD9, PCSA and Ago2, PCSA and CD9, Ago2 and CD9, or all of PCSA, Ago2 and CD9. Another general vesicle marker such as in **Table 3,** e.g., a tetraspanin such as CD63 or CD81 can be substituted for or used in addition to CD9. Such multiple biomarkers can be used to identify a microvesicle population having a certain origin. E.g., PCSA can identify prostate-derived vesicles while CD9 identifies vesicles apart from cellular debris. PCSA, PSMA, PSCA, KLK2 or PBP (prostate binding protein) can be used as a biomarker to characterize a prostate cancer.

[00446] The one or more nucleic acid biomarker may comprise one or more nucleic acid disclosed herein, such as in **Table 5.** In an embodiment, the one or more nucleic acid comprises one or more microRNA. For example, the one or more microRNA can be selected from 1, 2, 3, 4, 5 or 6 of miR-22, miR-16, miR-148a, miR-92a, miR-451, and let7a. In an embodiment, the one or more protein biomarker comprises PCSA and Ago2; and the one or more nucleic acid biomarker comprises miR-22. In another embodiment, the one or more protein biomarker comprises PCSA and/or CD9; and the one or more nucleic acid biomarker comprises miR-22. The method can be used to characterize a cancer such as a prostate cancer, e.g., to distinguish a cancer sample from a non-cancer sample.

[00447] In other embodiments, the one or more nucleic acid comprises mRNA. mRNA can be assessed as payload within microvesicles. For example, the one or more nucleic acid biomarker comprises a messenger RNA (mRNA) selected from **Table 5.** The mRNA may also be selected from any of **Tables 22-24.** In some embodiments, the one or more protein biomarker comprises PCSA; and the one or more nucleic acid biomarker

PREOMICS EXHIBIT 1002
Page 1058 of 1861

comprises a messenger RNA (mRNA) selected from any of **Tables 22-24.** The method can be used to characterize a cancer such as a prostate cancer, e.g., to distinguish a cancer sample from a non- cancer sample.

[00448] The level of the one or more one or more nucleic acid biomarker can be normalized to the level of the one or more protein biomarker. In an embodiment, the biosignature comprises a score calculated from a ratio of the level of the one or more protein biomarker and one or more nucleic acid biomarker. For example, the level of the nucleic acids can be divided by the level of the proteins.

[00449] The score can be calculated from multiple proteins and multiple nucleic acids. In an embodiment, the one or more protein biomarker comprises PCSA and PSMA and the one or more nucleic acid biomarker comprises miR-22 and let7a. The method is used to characterize a prostate cancer, e.g., to distinguish a prostate cancer sample from a non-prostate cancer sample. The score may comprise taking the sum of: a) a first multiple of the level of miR-22 payload in the microvesicle subpopulation divided by the level of PCSA protein associated with the microvesicle subpopulation; b) a second multiple of the level of let7a payload in the microvesicle subpopulation divided by the level of PCSA protein associated with the microvesicle subpopulation; and c) a third multiple of the level of PSMA protein associated with the microvesicle subpopulation. The first, second and third multiples can be chosen to maximize the ability of the method to distinguish the prostate cancer. For example, the multiple can be about 0.0001, 0.001, 0.01, 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 100, 1000 or 10000. In an embodiment, the first multiple is 10, the second multiple is 10, and the third multiple is 1. The score can be an average of the sum as:

Score =*Average(* 10*miR22/PCSA MFI, 10*let-7a/PCSA MFI, PSMA MFI)

[00450] One of skill will appreciate that calculating the score may comprise a monotonic transformation of the sum. A similar scoring equation can be developed for other biomarkers in other settings, such as using alternate biomarkers to characterize other cancers.

[00451] By selecting a proper reference sample for comparison, the biosignatures identified can provide a diagnostic readout (e.g., reference sample is normal or non-disease), prognostic (e.g., reference sample is for poor or good disease outcome, aggressiveness or the like), or theranostic (e.g., reference sample is from a cohort responsive or non-responsive to selected treatment).

[00452] Additional biomarkers that can be used in the methods of the invention include those disclosed in International Patent Application PCT/US2012/025741, filed February 17, 2012; International Patent Application PCT/US20 11/048327, filed August 18, 201 1; International Patent Application PCT/ US201 1/026750, filed March 1, 201 1; and International Patent Application PCT/US201 1/03 1479, filed April 6, 201 1; each ofwhich is incorporated by reference herein in its entirety.

[00453] ***Gene Fusions***

[00454] The one or more biomarkers assessed of vesicle, can be a gene fusion. A fusion gene is a hybrid gene created by the juxtaposition of two previously separate genes. This can occur by chromosomal translocation or inversion, deletion or via trans-splicing. The resulting fusion gene can cause abnormal temporal and spatial expression of genes, such as leading to abnormal expression ofcell growth factors, angiogenesis factors, tumor promoters or other factors contributing to the neoplastic transformation of the cell and the creation of a tumor. Such fusion genes can be oncogenic due to the juxtaposition of: 1) a strong promoter region of one gene next to the coding region of a cell growth factor, tumor promoter or other gene promoting oncogenesis leading to

PREOMICS EXHIBIT 1002
Page 1059 of 1861

elevated gene expression, or 2) due to the fusion of coding regions of two different genes, giving rise to a chimeric gene and thus a chimeric protein with abnormal activity.

[00455] An example of a fusion gene is BCR-ABL, a characteristic molecular aberration in ~90% of chronic myelogenous leukemia (CML) and in a subset of acute leukemias *{Kurzrock et al, Annals of Internal Medicine 2003; 138(10):819-830).* The BCR-ABL results from a translocation between chromosomes 9 and 22. The translocation brings together the 5' region of the BCR gene and the 3' region of ABL1, generating a chimeric BCR-ABL1 gene, which encodes a protein with constitutively active tyrosine kinase activity (Mittleman et al., Nature *Reviews Cancer* 2007; 7(4):233-245). The aberrant tyrosine kinase activity leads to de-regulated cell signaling, cell growth and cell survival, apoptosis resistance and growth factor independence, all of which contribute to the pathophysiology of leukemia (Kurzrock et al., *Annals of Internal Medicine* 2003; 138(10):819-830).

[00456] Another fusion gene is IGH-MYC, a defining feature of ~80% of Burkitt's lymphoma (Ferry et al. *Oncologist* 2006; 11(4):375-83). The causal event for this is a translocation between chromosomes 8 and 14, bringing the c-Myc oncogene adjacent to the strong promoter of the immunoglobin heavy chain gene, causing c-*myc* overexpression (Mittleman et al., *Nature Reviews Cancer* 2007; 7(4):233-245). The *c-myc* rearrangement is a pivotal event in lymphomagenesis as it results in a perpetually proliferative state. It has wide ranging effects on progression through the cell cycle, cellular differentiation, apoptosis, and cell adhesion (Ferry et al. *Oncologist* 2006; 11(4):375-83).

[00457] A number of recurrent fusion genes have been catalogued in the Mittleman database (cgap.nci.nih.gov/Chromosomes/Mitelman) and can be assessed in a vesicle, and used to characterize a phenotype. The gene fusion can be used to characterize a hematological malignancy or epithelial tumor. For example, TMPRSS2-ERG, TMPRSS2-ETV and SLC45A3-ELK4 fusions can be detected and used to characterize prostate cancer; and ETV6-NTRK3 and ODZ4-NRG1 for breast cancer.

[00458] Furthermore, assessing the presence or absence, or expression level of a fusion gene can be used to diagnosis a phenotype such as a cancer as well as a monitoring a therapeutic response to selecting a treatment. For example, the presence of the BCR-ABL fusion gene is a characteristic not only for the diagnosis of CML, but is also the target of the Novartis drug Imatinib mesylate (Gleevec), a receptor tyrosine kinase inhibitor, for the treatment of CML. Imatinib treatment has led to molecular responses (disappearance of BCR-ABL+ blood cells) and improved progression-free survival in BCR-ABL+ CML patients *(Kantarjian et al., Clinical Cancer Research 2007; 13(4): 1089-1097).*

[00459] Assessing a vesicle for the presence, absence, or expression level of a gene fusion can be of by assessing a heterogeneous population of vesicles for the presence, absence, or expression level of a gene fusion. Alternatively, the vesicle that is assessed can be derived from a specific cell type, such as cell-of-origin specific vesicle, as described above. Illustrative examples of use of fusions that can be assessed to characterize a phenotype include those described in International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein.

[00460] ***Gene-Associated MiRNA Biomarkers***

[00461] Illustrative examples of use of miRNA biomarkers known to interact with certain transcripts and that can be assessed to characterize a phenotype include those described in International Patent Application Serial

PREOMICS EXHIBIT 1002
Page 1060 of 1861

No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein.

[00462] ***Nucleic acid - Protein Complex Biomarkers***

[00463] MicroRNAs in human plasma have been found associated with circulating microvesicles, Argonaute proteins, and HDL and LDL complexes. See, e.g., Arroyo et al., Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA. 201 1. 108:5003-08. Epub 201 1 Mar 7; Collino et al., Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. PLOS One. 2010 5(7):el 1803. The Argonaute family of proteins plays a role in RNA interference (RNAi) gene silencing. Argonaute proteins bind short RNAs such as microRNAs (miRNAs) or short interfering RNAs (siRNAs), and repress the translation of their complementary mRNAs. They are also involved in transcriptional gene silencing (TGS), in which short RNAs known as antigene RNAs or agRNAs direct the transcriptional repression of complementary promoter regions. Argonaute family members include Argonaute 1 ("eukaryotic translation initiation factor 2C, 1", EIF2C1, AGOl), Argonaute 2 ("eukaryotic translation initiation factor 2C, 2", EIF2C2, AG02), Argonaute 3 ("eukaryotic translation initiation factor 2C, 3", EIF2C3, AG03), and Argonaute 4 ("eukaryotic translation initiation factor 2C, 4", EIF2C4, AG04). Several Argonaute isotypes have been identified. Argonaute 2 is an effector protein within the RNA-Induced Silencing Complex (RISC) where it plays a role in the silencing of target messenger RNAs in the microRNA silencing pathway.

[00464] The protein GW1 82 associates with microvesicles and also has the capacity to bind all human Argonaute proteins (e.g., Agol, Ago2, Ago3, Ago4) and their associated miRNAs. See, e.g., Gibbings et al., Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity, Nat Cell Biol 2009 11:1143-1 149. Epub 2009 Aug 16; Lazzaretti et al., The C-terminal domains of human TNRC6A, TNRC6B, and TNRC6C silence bound transcripts independently of Argonaute proteins. RNA. 2009 15: 1059-66. Epub 2009 Apr 2 1. GW1 82, which is encoded by the TNRC6A gene (trinucleotide repeat containing 6A), functions in post-transcriptional gene silencing through the RNA interference (RNAi) and microRNA pathways. TNRC6B and TNRC6C are also members of the trinucleotide repeat containing 6 family and play similar roles in gene silencing. GW1 82 associates with mRNAs and Argonaute proteins in cytoplasmic bodies known as GW-bodies or P-bodies. GW1 82 is involved in miRNA-dependent repression of translation and for siRNA-dependent endonucleolytic cleavage of complementary mRNAs by argonaute family proteins.

[00465] In an aspect, the invention provides a method of characterizing a phenotype comprising analyzing nucleic acid - protein complex biomarkers. As used herein, a nucleic acid - protein complex comprises at least one nucleic acid and at least one protein, and can also include other components such as lipids. A nucleic acid - protein complex can be associated with a vesicle. In an embodiment, RNA - protein complexes are isolated and the levels of the associated RNAs are assessed, wherein the levels are used for characterizing the phenotype, e.g., providing a diagnosis, prognosis, theranosis, or other phenotype as described herein. The RNA can be microRNA. MicroRNAs have been found associated with vesicles and proteins. In some cases, this association may serve to protect miRNAs from degradation via RNAses or other factors. Content of various populations of microRNA can be assessed in a sample, including without limitation vesicle associated miRs, Ago-associated miRs, cell-of-origin vesicle associated miRs, circulating Ago-bound miRs, circulating HDL-bound miRs, and the total miR content.

PREOMICS EXHIBIT 1002
Page 1061 of 1861

[00466] The protein biomarker used to isolate the complexes can be one or more Argonaute protein, or other protein that associates with Argonaute family members. These include without limitation the Argonaute proteins Agol, Ago2, Ago3, Ago4, and various isoforms thereof. The protein biomarker can be GW1 82 (TNRC6A), TNRC6B and/or TNRC6C. The protein biomarker can be a protein associated with a P-body or a GW-body, such as SW182, an argonaute, decapping enzyme or RNA helicase. See, e.g., Kulkarni et al. On track with P-bodies. Biochem Soc Trans 2010, 38:242-251 . The protein biomarker can also be one or more of HNRNPA2B1 (Heterogeneous nuclear ribonucleoprotein a2/bl), HNRPAB (Heterogeneous nuclear ribonucleoprotein A/B), ILF2 (Interleukin enhancer binding factor 2, 45 kda), NCL (Nucleolin), NPM 1 (Nucleophosmin (nucleolar phosphoprotein b23, numatrin)), RPL10A (Ribosomal protein 110a), RPL5 (Ribosomal protein 15), RPLP1 (Ribosomal protein, large, pi), RPS12 (Ribosomal protein sl2), RPS19 (Ribosomal protein sl9), SNRPG (Small nuclear ribonucleoprotein polypeptide g), TROVE2 (Trove domain family, member 2). See Wang et al., Export of microRNAs and microRNA-protective protein by mammalian cells. Nucleic Acids Res. 38:7248-59. Epub 2010 Jul 7. The protein biomarker can also be an apolipoprotem, which are proteins that bind to lipids (oil-soluble substances such as fat and cholesterol) to form lipoproteins, which transport the lipids through the lymphatic and circulatory systems. See Vickers et al., MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins, Nat Cell Biol 201 1 13:423-33, Epub 201 1 Mar 20. The apolipoprotem can be apolipoprotem A (including apo A-I, apo A-II, apo A-IV, and apo A-V), apolipoprotem B (including apo B48 and apo B100), apolipoprotem C (including apo C-I, apo C-II, apo C-III, and apo C-IV), apolipoprotem D (ApoD), apolipoprotem E (ApoE), apolipoprotem H (ApoH), or a combination thereof. The apolipoprotem can be apolipoprotem L, including APOL1, APOL2, APOL3, APOL4, APOL5, APOL6, APOLD1, or a combination thereof. Apolipoprotem L (Apo L) belongs to the high density lipoprotein family that plays a central role in cholesterol transport. The protein biomarker can be a component of a lipoprotein, such as a component of a chylomicron, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and/or high density lipoprotein (HDL). In an embodiment, the protein biomarker is a component of a LDL or HDL. The component can be ApoE. The component can be ApoAl . The protein biomarker can be a general vesicle marker, such as a tetraspanin or other protein listed in **Table 3,** including without limitation CD9, CD63 and/or CD81 . The protein biomarker can be a cancer marker such as EpCam, B7H3 and/or CD24. The protein biomarker can be a tissue specific biomarker, such as the prostate biomarkers PSCA, PCSA and/or PSMA. Combinations of these or other useful protein biomarkers can be used to isolate specific populations of complexes of interest.

[00467] The nucleic acid - protein complexes can be isolated by using a binding agent to one or more component of the complexes. Various techniques for isolating proteins are known to those of skill in the art and/or presented herein, including without limitation affinity isolation, immunocapture, immunoprecipitation, and flow cytometry. The binding agent can be any appropriate binding agent, including those described herein such as the one or more binding agent comprises a nucleic acid, DNA molecule, RNA molecule, antibody, antibody fragment, aptamer, peptoid, zDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), lectin, peptide, dendrimer, membrane protein labeling agent, chemical compound, or a combination thereof. In an embodiment, the binding agent comprises an antibody, antibody conjugate, antibody fragment, and/or aptamer. For additional methods of assessing protein - nucleic acid complexes that can be used with the subject invention, see also Wang et al., Export of microRNAs and microRNA-protective protein by mammalian cells.

PREOMICS EXHIBIT 1002
Page 1062 of 1861

Nucleic Acids Res. 38:7248-59. Epub 2010 Jul 7; Keene et al., RIP-Chip: the isolation and identification of niRNAs, microRNAs and protein components of ribonucleoprotein complexes from cell extracts. Nat Protoc 2006 1:302-07; Hafner, Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell 2010 141:129-41.

[00468] The present invention further provides a method of identifying miRNAs that are found in complex with proteins. In one embodiment, a population of protein - nucleic acid complexes is isolated as described above. The miRNA content of the population is assessed. This method can be used on various samples of interest (e.g., diseased, non-diseased, responder, non-responder) and the miRNA content in the samples can be compared to identify miRNAs that differentiate between the samples. Methods of detecting miRNA are provided herein (arrays, per, etc). The identified miRNAs can be used to characterize a phenotype according to the methods herein. For example, the samples used for discovery can be cancer and non-cancer plasma samples. Protein-complexed miRNAs can be identified that distinguish between the cancer and non-cancer samples, and the distinguishing miRNAs can be assessed in order to detect a cancer in a plasma sample.

[00469] The present invention also provides a method of distinguishing microRNA payload within vesicles by removing non-payload miRs from a vesicle-containing sample, then assessing the miR content within the vesicles. miRs can be removed from the sample using RNAses or other entities that degrade miRNA. In some embodiments, the sample is treated with an agent to remove microRNAs from protein complexes prior to the RNAse treatment. The agent can be an enzyme that degrades protein, e.g., a proteinase such as Proteinase K or Trypsin, or any other appropriate enzyme. The method can be used to characterize a phenotype according to the methods herein by assessing the microRNA fraction contained with vesicles apart from free miRNA or miRNA in circulating protein complexes.

Biomarker Detection

[00470] A biosignature can be detected qualitatively or quantitatively by detecting a presence, level or concentration of a circulating biomarker, e.g., a microRNA, protein, vesicle or other biomarker, as disclosed herein. These biosignature components can be detected using a number of techniques known to those of skill in the art. For example, a biomarker can be detected by microarray analysis, polymerase chain reaction (PCR) (including PCR-based methods such as real time polymerase chain reaction (RT-PCR), quantitative real time polymerase chain reaction (Q-PCR/qPCR) and the like), hybridization with allele-specific probes, enzymatic mutation detection, ligation chain reaction (LCR), oligonucleotide ligation assay (OLA), flow-cytometric heteroduplex analysis, chemical cleavage of mismatches, mass spectrometry, nucleic acid sequencing, single strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), restriction fragment polymorphisms, serial analysis of gene expression (SAGE), or combinations thereof. A biomarker, such as a nucleic acid, can be amplified prior to detection. A biomarker can also be detected by immunoassay, immunoblot, immunoprecipitation, enzyme-linked immunosorbent assay (ELISA; EIA), radioimmunoassay (RIA), flow cytometry, or electron microscopy (EM).

[00471] Biosignatures can be detected using capture agents and detection agents, as described herein. A capture agent can comprise an antibody, aptamer or other entity which recognizes a biomarker and can be used for capturing the biomarker. Biomarkers that can be captured include circulating biomarkers, e.g., a protein, nucleic acid, lipid or biological complex in solution in a bodily fluid. Similarly, the capture agent can be used for capturing a vesicle. A detection agent can comprise an antibody or other entity which recognizes a biomarker

PREOMICS EXHIBIT 1002
Page 1063 of 1861

and can be used for detecting the biomarker vesicle, or which recognizes a vesicle and is useful for detecting a vesicle. In some embodiments, the detection agent is labeled and the label is detected, thereby detecting the biomarker or vesicle. The detection agent can be a binding agent, e.g., an antibody or aptamer. In other embodiments, the detection agent comprises a small molecule such as a membrane protein labeling agent. See, e.g., the membrane protein labeling agents disclosed in Alroy et al., US. Patent Publication US 2005/0158708. In an embodiment, vesicles are isolated or captured as described herein, and one or more membrane protein labeling agent is used to detect the vesicles. In many cases, the antigen or other vesicle-moiety that is recognized by the capture and detection agents are interchangeable. As a non-limiting example, consider a vesicle having a cell-of-origin specific antigen on its surface and a cancer-specific antigen on its surface. In one instance, the vesicle can be captured using an antibody to the cell-of-origin specific antigen, e.g., by tethering the capture antibody to a substrate, and then the vesicle is detected using an antibody to the cancer-specific antigen, e.g., by labeling the detection antibody with a fluorescent dye and detecting the fluorescent radiation emitted by the dye. In another instance, the vesicle can be captured using an antibody to the cancer specific antigen, e.g., by tethering the capture antibody to a substrate, and then the vesicle is detected using an antibody to the cell-of-origin specific antigen, e.g., by labeling the detection antibody with a fluorescent dye and detecting the fluorescent radiation emitted by the dye.

[00472] In some embodiments, a same biomarker is recognized by both a capture agent and a detection agent. This scheme can be used depending on the setting. In one embodiment, the biomarker is sufficient to detect a vesicle of interest, e.g., to capture cell-of-origin specific vesicles. In other embodiments, the biomarker is multifunctional, e.g., having both cell-of-origin specific and cancer specific properties. The biomarker can be used in concert with other biomarkers for capture and detection as well.

[00473] One method of detecting a biomarker comprises purifying or isolating a heterogeneous population of vesicles from a biological sample, as described above, and performing a sandwich assay. A vesicle in the population can be captured with a capture agent. The capture agent can be a capture antibody, such as a primary antibody. The capture antibody can be bound to a substrate, for example an array, well, or particle. The captured or bound vesicle can be detected with a detection agent, such as a detection antibody. For example, the detection antibody can be for an antigen of the vesicle. The detection antibody can be directly labeled and detected. Alternatively, the detection agent can be indirectly labeled and detected, such as through an enzyme linked secondary antibody that can react with the detection agent. A detection reagent or detection substrate can be added and the reaction detected, such as described in PCT Publication No. WO2009092386. In an illustrative example wherein the capture agent binds Rab-5b and the detection agent binds or detects CD63 or caveolin-1, the capture agent can be an anti-Rab 5b antibody and the detection agent can be an anti-CD63 or anti-caveolin-1 antibody. In some embodiments, the capture agent binds CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, or 5T4. For example, the capture agent can be an antibody to CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, or 5T4. The capture agent can also be an antibody to MFG-E8, Annexin V, Tissue Factor, DR3, STEAP, epha2, TMEM21 1, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, or TETS. The detection agent can be an agent that binds or detects CD63, CD9, CD81, B7H3, or EpCam, such as a detection antibody or aptamer to CD63, CD9, CD81, B7H3, or EpCam. Various combinations of capture and/or detection agents can be used in concert. In an embodiment, the capture agents comprise PCSA, PSMA, B7H3 and optionally EpCam, and the detection agents comprise one or

PREOMICS EXHIBIT 1002
Page 1064 of 1861

more general vesicle biomarker, e.g., a tetraspanin such as CD9, CD63 and CD81 . In another embodiment, the capture agents comprise TMEM21 1 and CD24, and the detection agents comprise one or more tetraspanin such as CD9, CD63 and CD81 . In another embodiment, the capture agents comprise CD66 and EpCam, and the detection agents comprise one or more tetraspanin such as CD9, CD63 and CD81 . The capture agent and/or detection agent can be to an antigen comprising one or more of CD9, Erb2, Erb4, CD81, Erb3, MUC16, CD63, DLL4, HLA-Drpe, B7H3, IFNAR, 5T4, PCSA, MICB, PSMA, MFG-E8, Mucl, PSA, Muc2, Unc93a, VEGFR2, EpCAM, VEGF A, TMPRSS2, RAGE*, PSCA, CD40, Mucl7, IL-17-RA, and CD80. For example, capture agent and/or detection agent can be to one or more of CD9, CD63, CD81, B7H3, PCSA, MFG-E8, MUC2, EpCam, RAGE and Mucl7. Increasing numbers of such tetraspanins and/or other general vesicle markers can improve the detection signal in some cases. Proteins or other circulating biomarkers can also be detected using sandwich approaches. The captured vesicles can be collected and used to analyze the payload contained therein, e.g., mRNA, microRNAs, DNA and soluble protein.

[00474] In some embodiments, the capture agent binds or targets EpCam, B7H3, RAGE or CD24, and the one or more biomarkers detected on the vesicle are CD9 and/or CD63. In one embodiment, the capture agent binds or targets EpCam, and the one or more biomarkers detected on the vesicle are CD9, EpCam and/or CD81 . The single capture agent can be selected from CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, or 5T4. The single capture agent can also be an antibody to DR3, STEAP, epha2, TMEM21 1, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, MFG-E8, TF, Annexin V or TETS. In some embodiments, the single capture agent is selected from PCSA, PSMA, B7H3, CD81, CD9 and CD63.

[00475] In other embodiments, the capture agent targets PCSA, and the one or more biomarkers detected on the captured vesicle are B7H3 and/or PSMA. In other embodiments, the capture agent targets PSMA, and the one or more biomarkers detected on the captured vesicle are B7H3 and/or PCSA. In other embodiments, the capture agent targets B7H3, and the one or more biomarkers detected on the captured vesicle are PSMA and/or PCSA. In yet other embodiments, the capture agent targets CD63 and the one or more biomarkers detected on the vesicle are CD81, CD83, CD9 and/or CD63. The different capture agent and biomarker combinations disclosed herein can be used to characterize a phenotype, such as detecting, diagnosing or prognosing a disease, e.g., a cancer. In some embodiments, vesicles are analyzed to characterize prostate cancer using a capture agent targeting EpCam and detection of CD9 and CD63; a capture agent targeting PCSA and detection of B7H3 and PSMA; or a capture agent of CD63 and detection of CD81 . In other embodiments, vesicles are used to characterize colon cancer using capture agent targeting CD63 and detection of CD63, or a capture agent targeting CD9 coupled with detection of CD63 . One of skill will appreciate that targets of capture agents and detection agents can be used interchangeably. In an illustrative example, consider a capture agent targeting PCSA and detection agents targeting B7H3 and PSMA. Because all of these markers are useful for detecting PCa derived vesicles, B7H3 or PSMA could be targeted by the capture agent and PCSA could be recognized by a detection agent. For example, in some embodiments, the detection agent targets PCSA, and one or more biomarkers used to capture the vesicle comprise B7H3 and/or PSMA. In other embodiments, the detection agent targets PSMA, and the one or more biomarkers used to capture the vesicle comprise B7H3 and/or PCSA. In other embodiments, the detection agent targets B7H3, and the one or more biomarkers used to capture the vesicle comprise PSMA and/or PCSA. In some embodiments, the invention provides a method of detecting prostate cancer cells in bodily fluid using capture agents and/or detection agents to PSMA, B7H3 and/or PCSA.

PREOMICS EXHIBIT 1002
Page 1065 of 1861

The bodily fluid can comprise blood, including serum or plasma. The bodily fluid can comprise ejaculate or sperm. In further embodiments, the methods of detecting prostate cancer further use capture agents and/or detection agents to CD81, CD83, CD9 and/or CD63. The method further provides a method of characterizing a GI disorder, comprising capturing vesicles with one or more of DR3, STEAP, epha2, TMEM21 1, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, and TETS, and detecting the captured vesicles with one or more general vesicle antigen, such as CD81, CD63 and/or CD9. Additional agents can improve the test performance, e.g., improving test accuracy or AUC, either by providing additional biological discriminatory power and/or by reducing experimental noise.

[00476] Techniques of detecting biomarkers for use with the invention include the use of a planar substrate such as an array (e.g., biochip or microarray), with molecules immobilized to the substrate as capture agents that facilitate the detection of a particular biosignature. The array can be provided as part of a kit for assaying one or more biomarkers or vesicles. A molecule that identifies the biomarkers described above and shown in **Figs. 1 or 3-60** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein, can be included in an array for detection and diagnosis of diseases including presymptomatic diseases. In some embodiments, an array comprises a custom array comprising biomolecules selected to specifically identify biomarkers of interest. Customized arrays can be modified to detect biomarkers that increase statistical performance, e.g., additional biomolecules that identifies a biosignature which lead to improved cross-validated error rates in multivariate prediction models (e.g., logistic regression, discriminant analysis, or regression tree models). In some embodiments, customized array(s) are constructed to study the biology of a disease, condition or syndrome and profile biosignatures in defined physiological states. Markers for inclusion on the customized array be chosen based upon statistical criteria, e.g., having a desired level of statistical significance in differentiating between phenotypes or physiological states. In some embodiments, standard significance of p-value = 0.05 is chosen to exclude or include biomolecules on the microarray. The p-values can be corrected for multiple comparisons. As an illustrative example, nucleic acids extracted from samples from a subject with or without a disease can be hybridized to a high density microarray that binds to thousands of gene sequences. Nucleic acids whose levels are significantly different between the samples with or without the disease can be selected as biomarkers to distinguish samples as having the disease or not. A customized array can be constructed to detect the selected biomarkers. In some embodiments, customized arrays comprise low density microarrays, which refer to arrays with lower number of addressable binding agents, e.g., tens or hundreds instead of thousands. Low density arrays can be formed on a substrate. In some embodiments, customizable low density arrays use PCR amplification in plate wells, e.g., TaqMan® Gene Expression Assays (Applied Biosystems by Life Technologies Corporation, Carlsbad, CA).

[00477] A planar array generally contains addressable locations (e.g., pads, addresses, or micro-locations) of biomolecules in an array format. The size of the array will depend on the composition and end use of the array. Arrays can be made containing from 2 different molecules to many thousands. Generally, the array comprises from two to as many as 100,000 or more molecules, depending on the end use of the array and the method of manufacture. A microarray for use with the invention comprises at least one biomolecule that identifies or captures a biomarker present in a biosignature of interest, e.g., a microRNA or other biomolecule or vesicle that

PREOMICS EXHIBIT 1002
Page 1066 of 1861

makes up the biosignature. In some arrays, multiple substrates are used, either of different or identical compositions. Accordingly, planar arrays may comprise a plurality of smaller substrates.

[00478] The present invention can make use of many types of arrays for detecting a biomarker, e.g., a biomarker associated with a biosignature of interest. Useful arrays or microarrays include without limitation DNA microarrays, such as cDNA microarrays, oligonucleotide microarrays and SNP microarrays, microRNA arrays, protein microarrays, antibody microarrays, tissue microarrays, cellular microarrays (also called transfection microarrays), chemical compound microarrays, and carbohydrate arrays (glycoarrays). These arrays are described in more detail above. In some embodiments, microarrays comprise biochips that provide high-density immobilized arrays of recognition molecules (e.g., antibodies), where biomarker binding is monitored indirectly (e.g., via fluorescence). **FIG. 2A** shows an illustrative configuration in which capture antibodies against a vesicle antigen of interest are tethered to a surface. The captured vesicles are then detected using detector antibodies against the same or different vesicle antigens of interest. The capture antibodies can be substituted with tethered aptamers as available and desirable. Fluorescent detectors are shown. Other detectors can be used similarly, e.g., enzymatic reaction, detectable nanoparticles, radiolabels, and the like. In other embodiments, an array comprises a format that involves the capture of proteins by biochemical or intermolecular interaction, coupled with detection by mass spectrometry (MS). The vesicles can be eluted from the surface and the payload therein, e.g., microRNA, can be analyzed.

[00479] An array or microarray that can be used to detect one or more biomarkers of a biosignature can be made according to the methods described in U.S. Pat. Nos. 6,329,209; 6,365,418; 6,406,921; 6,475,808; and 6,475,809, and U.S. Patent Application Ser. No. 10/884,269, each of which is herein incorporated by reference in its entirety. Custom arrays to detect specific selections of sets of biomarkers described herein can be made using the methods described in these patents. Commercially available microarrays can also be used to carry out the methods of the invention, including without limitation those from Affymetrix (Santa Clara, CA), Illumina (San Diego, CA), Agilent (Santa Clara, CA), Exiqon (Denmark), or Invitrogen (Carlsbad, CA). Custom and/or commercial arrays include arrays for detection proteins, nucleic acids, and other biological molecules and entities (e.g., cells, vesicles, virii) as described herein.

[00480] In some embodiments, molecules to be immobilized on an array comprise proteins or peptides. One or more types of proteins may be immobilized on a surface. In certain embodiments, the proteins are immobilized using methods and materials that minimize the denaturing of the proteins, that minimize alterations in the activity of the proteins, or that minimize interactions between the protein and the surface on which they are immobilized.

[00481] Array surfaces useful may be of any desired shape, form, or size. Non-limiting examples of surfaces include chips, continuous surfaces, curved surfaces, flexible surfaces, films, plates, sheets, or tubes. Surfaces can have areas ranging from approximately a square micron to approximately 500 cm^2. The area, length, and width of surfaces may be varied according to the requirements of the assay to be performed. Considerations may include, for example, ease of handling, limitations of the material(s) of which the surface is formed, requirements of detection systems, requirements of deposition systems (e.g., arrayers), or the like.

[00482] In certain embodiments, it is desirable to employ a physical means for separating groups or arrays of binding islands or immobilized biomolecules: such physical separation facilitates exposure of different groups or arrays to different solutions of interest. Therefore, in certain embodiments, arrays are situated within

PREOMICS EXHIBIT 1002
Page 1067 of 1861

microwell plates having any number of wells. In such embodiments, the bottoms of the wells may serve as surfaces for the formation of arrays, or arrays may be formed on other surfaces and then placed into wells. In certain embodiments, such as where a surface without wells is used, binding islands may be formed or molecules may be immobilized on a surface and a gasket having holes spatially arranged so that they correspond to the islands or biomolecules may be placed on the surface. Such a gasket is preferably liquid tight. A gasket may be placed on a surface at any time during the process of making the array and may be removed if separation of groups or arrays is no longer necessary.

[00483] In some embodiments, the immobilized molecules can bind to one or more biomarkers or vesicles present in a biological sample contacting the immobilized molecules. In some embodiments, the immobilized molecules modify or are modified by molecules present in the one or more vesicles contacting the immobilized molecules. Contacting the sample typically comprises overlaying the sample upon the array.

[00484] Modifications or binding of molecules in solution or immobilized on an array can be detected using detection techniques known in the art. Examples of such techniques include immunological techniques such as competitive binding assays and sandwich assays; fluorescence detection using instruments such as confocal scanners, confocal microscopes, or CCD-based systems and techniques such as fluorescence, fluorescence polarization (FP), fluorescence resonant energy transfer (FRET), total internal reflection fluorescence (TIRF), fluorescence correlation spectroscopy (FCS); colorimetric/spectrometric techniques; surface plasmon resonance, by which changes in mass of materials adsorbed at surfaces are measured; techniques using radioisotopes, including conventional radioisotope binding and scintillation proximity assays (SPA); mass spectroscopy, such as matrix-assisted laser desorption/ionization mass spectroscopy (MALDI) and MALDI-time of flight (TOF) mass spectroscopy; ellipsometry, which is an optical method of measuring thickness of protein films; quartz crystal microbalance (QCM), a very sensitive method for measuring mass of materials adsorbing to surfaces; scanning probe microscopies, such as atomic force microscopy (AFM), scanning force microscopy (SFM) or scanning electron microscopy (SEM); and techniques such as electrochemical, impedance, acoustic, microwave, and IR/Raman detection. See, e.g., *Mere L, et al, "Miniaturized FRET assays and microfluidics: key components for ultra-high-throughput screening, "Drug Discovery Today 4(8):363-369 (1999),* and references cited therein; *Lakowicz J R, Principles of Fluorescence Spectroscopy, 2nd Edition, Plenum Press (1999), or Jain KK: Integrative Omics, Pharmacoproteomics, and Human Body Fluids. In: Thongboonkerd V, ed., ed. Proteomics of Human Body Fluids: Principles, Methods and Applications. Volume 1: Totowa, N.J.: Humana Press, 2007,* each of which is herein incorporated by reference in its entirety.

[00485] Microarray technology can be combined with mass spectroscopy (MS) analysis and other tools. Electrospray interface to a mass spectrometer can be integrated with a capillary in a microfluidics device. For example, one commercially available system contains eTag reporters that are fluorescent labels with unique and well-defined electrophoretic mobilities; each label is coupled to biological or chemical probes via cleavable linkages. The distinct mobility address of each eTag reporter allows mixtures of these tags to be rapidly deconvoluted and quantitated by capillary electrophoresis. This system allows concurrent gene expression, protein expression, and protein function analyses from the same sample *Jain KK: Integrative Omics, Pharmacoproteomics, and Human Body Fluids. In: Thongboonkerd V, ed., ed. Proteomics of Human Body Fluids: Principles, Methods and Applications. Volume 1: Totowa, N.J.: Humana Press, 2007, which* is herein incorporated by reference in its entirety.

PREOMICS EXHIBIT 1002
Page 1068 of 1861

[00486] A biochip can include components for a microfluidic or nanofluidic assay. A microfluidic device can be used for isolating or analyzing biomarkers, such as determining a biosignature. Microfluidic systems allow for the miniaturization and compartmentalization of one or more processes for isolating, capturing or detecting a vesicle, detecting a microRNA, detecting a circulating biomarker, detecting a biosignature, and other processes. The microfluidic devices can use one or more detection reagents in at least one aspect of the system, and such a detection reagent can be used to detect one or more biomarkers. In one embodiment, the device detects a biomarker on an isolated or bound vesicle. Various probes, antibodies, proteins, or other binding agents can be used to detect a biomarker within the microfluidic system. The detection agents may be immobilized in different compartments of the microfluidic device or be entered into a hybridization or detection reaction through various channels of the device.

[00487] A vesicle in a microfluidic device can be lysed and its contents detected within the microfluidic device, such as proteins or nucleic acids, e.g., DNA or RNA such as miRNA or mRNA. The nucleic acid may be amplified prior to detection, or directly detected, within the microfluidic device. Thus microfluidic system can also be used for multiplexing detection of various biomarkers. In an embodiment, vesicles are captured within the microfluidic device, the captured vesicles are lysed, and a biosignature of microRNA from the vesicle payload is determined. The biosignature can further comprise the capture agent used to capture the vesicle.

[00488] Novel nanofabrication techniques are opening up the possibilities for biosensing applications that rely on fabrication of high-density, precision arrays, e.g., nucleotide-based chips and protein arrays otherwise know as heterogeneous nanoarrays. Nanofluidics allows a further reduction in the quantity of fluid analyte in a microchip to nanoliter levels, and the chips used here are referred to as nanochips. (See, e.g., *Unger M et al, Biotechniques 1999; 27(5): 1008-14, Kartalov EP et al, Biotechniques 2006; 40(l):85-90,* each of which are herein incorporated by reference in their entireties.) Commercially available nanochips currently provide simple one step assays such as total cholesterol, total protein or glucose assays that can be run by combining sample and reagents, mixing and monitoring of the reaction. Gel-free analytical approaches based on liquid chromatography (LC) and nanoLC separations *(Cutillas et al. Proteomics, 2005;5:101-112 and Cutillas et al, Mol Cell Proteomics 2005;4:1038-1051,* each of which is herein incorporated by reference in its entirety) can be used in combination with the nanochips.

[00489] An array suitable for identifying a disease, condition, syndrome or physiological status can be included in a kit. A kit can include, as non-limiting examples, one or more reagents useful for preparing molecules for immobilization onto binding islands or areas of an array, reagents useful for detecting binding of a vesicle to immobilized molecules, and instructions for use.

[00490] Further provided herein is a rapid detection device that facilitates the detection of a particular biosignature in a biological sample. The device can integrate biological sample preparation with polymerase chain reaction (PCR) on a chip. The device can facilitate the detection of a particular biosignature of a vesicle in a biological sample, and an example is provided as described in *Pipper et al, Angewandte Chemie, 47(21), p. 3900-3904 (2008),* which is herein incorporated by reference in its entirety. A biosignature can be incorporated using micro-/nano-electrochemical system (MEMS/NEMS) sensors and oral fluid for diagnostic applications as described in *Li et al, Adv Dent Res 18(1): 3-5 (2005),* which is herein incorporated by reference in its entirety.

[00491] As an alternative to planar arrays, assays using particles, such as bead based assays as described herein, can be used in combination with flow cytometry. Multiparametric assays or other high throughput detection

PREOMICS EXHIBIT 1002
Page 1069 of 1861

assays using bead coatings with cognate ligands and reporter molecules with specific activities consistent with high sensitivity automation can be used. In a bead based assay system, a binding agent for a biomarker or vesicle, such as a capture agent (e.g. capture antibody), can be immobilized on an addressable microsphere. Each binding agent for each individual binding assay can be coupled to a distinct type of microsphere (i.e., microbead) and the assay reaction takes place on the surface of the microsphere, such as depicted in **FIG. 2B.** A binding agent for a vesicle can be a capture antibody coupled to a bead. Dyed microspheres with discrete fluorescence intensities are loaded separately with their appropriate binding agent or capture probes. The different bead sets carrying different binding agents can be pooled as necessary to generate custom bead arrays. Bead arrays are then incubated with the sample in a single reaction vessel to perform the assay. Examples of microfluidic devices that may be used, or adapted for use with the invention, include but are not limited to those described herein.

[00492] Product formation of the biomarker with an immobilized capture molecule or binding agent can be detected with a fluorescence based reporter system (see for example, **FIG. 2A-B).** The biomarker can either be labeled directly by a fluorophore or detected by a second fluorescently labeled capture biomolecule. The signal intensities derived from captured biomarkers can be measured in a flow cytometer. The flow cytometer can first identify each microsphere by its individual color code. For example, distinct beads can be dyed with discrete fluorescence intensities such that each bead with a different intensity has a different binding agent. The beads can be labeled or dyed with at least 2 different labels or dyes. In some embodiments, the beads are labeled with at least 3, 4, 5, 6, 7, 8, 9, or 10 different labels. The beads with more than one label or dye can also have various ratios and combinations of the labels or dyes. The beads can be labeled or dyed externally or may have intrinsic fluorescence or signaling labels.

[00493] The amount of captured biomarkers on each individual bead can be measured by the second color fluorescence specific for the bound target. This allows multiplexed quantitation of multiple targets from a single sample within the same experiment. Sensitivity, reliability and accuracy are compared or can be improved to standard microtiter ELISA procedures. An advantage of a bead-based system is the individual coupling of the capture biomolecule or binding agent for a vesicle to distinct microspheres provides multiplexing capabilities. For example, as depicted in **FIG. 2C,** a combination of 5 different biomarkers to be detected (detected by antibodies to antigens such as CD63, CD9, CD81, B7H3, and EpCam) and 20 biomarkers for which to capture a vesicle, (using capture antibodies, such as antibodies to CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, 5T4, and/or CD24) can result in approximately 100 combinations to be detected. As shown in **FIG. 2C** as "EpCam 2x," "CD63 2X," multiple antibodies to a single target can be used to probe detection against various epitopes. In another example, multiplex analysis comprises capturing a vesicle using a binding agent to CD24 and detecting the captured vesicle using a binding agent for CD9, CD63, and/or CD81. The captured vesicles can be detected using a detection agent such as an antibody. The detection agents can be labeled directly or indirectly, as described herein.

[00494] Multiplexing of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 different biomarkers may be performed. For example, an assay of a heterogeneous population of vesicles can be performed with a plurality of particles that are differentially labeled. There can be at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 differentially labeled particles. The particles may be externally labeled, such as with a tag, or they may be intrinsically labeled. Each differentially labeled particle

PREOMICS EXHIBIT 1002
Page 1070 of 1861

can be coupled to a capture agent, such as a binding agent, for a vesicle, resulting in capture of a vesicle. The multiple capture agents can be selected to characterize a phenotype of interest, including capture agents against general vesicle biomarkers, cell-of-origin specific biomarkers, and disease biomarkers. One or more biomarkers of the captured vesicle can then be detected by a plurality of binding agents. The binding agent can be directly labeled to facilitate detection. Alternatively, the binding agent is labeled by a secondary agent. For example, the binding agent may be an antibody for a biomarker on the vesicle. The binding agent is linked to biotin. A secondary agent comprises streptavidin linked to a reporter and can be added to detect the biomarker. In some embodiments, the captured vesicle is assayed for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 different biomarkers. For example, multiple detectors, i.e., detection of multiple biomarkers of a captured vesicle or population of vesicles, can increase the signal obtained, permitted increased sensitivity, specificity, or both, and the use of smaller amounts of samples. For example, detection with more than one general vesicle marker can improve the signal as compared to using a lesser number of detection markers, such as a single marker. To illustrate, detection of vesicles with labeled binding agents to two or three of CD9, CD63 and CD81 can improve the signal compared to detection with any one of the tetraspanins individually.

[00495] An immunoassay based method or sandwich assay can also be used to detect a biomarker of a vesicle. An example includes ELISA. A binding agent or capture agent can be bound to a well. For example an antibody to an antigen of a vesicle can be attached to a well. A biomarker on the captured vesicle can be detected based on the methods described herein. **FIG. 2A** shows an illustrative schematic for a sandwich-type of immunoassay. The capture antibody can be against a vesicle antigen of interest, e.g., a general vesicle biomarker, a cell-of-origin marker, or a disease marker. In the figure, the captured vesicles are detected using fluorescently labeled antibodies against vesicle antigens of interest. Multiple capture antibodies can be used, e.g., in distinguishable addresses on an array or different wells of an immunoassay plate. The detection antibodies can be against the same antigen as the capture antibody, or can be directed against other markers. The capture antibodies can be substituted with alternate binding agents, such as tethered aptamers or lectins, and/or the detector antibodies can be similarly substituted, e.g., with detectable (e.g., labeled) aptamers, lectins or other binding proteins or entities. In an embodiment, one or more capture agents to a general vesicle biomarker, a cell-of-origin marker, and/or a disease marker are used along with detection agents against general vesicle biomarker, such as tetraspanin molecules including without limitation one or more of CD9, CD63 and CD81.

[00496] **FIG. 2D** presents an illustrative schematic for analyzing vesicles according to the methods of the invention. Capture agents are used to capture vesicles, detectors are used to detect the captured vesicles, and the level or presence of the captured and detected antibodies is used to characterize a phenotype. Capture agents, detectors and characterizing phenotypes can be any of those described herein. For example, capture agents include antibodies or aptamers tethered to a substrate that recognize a vesicle antigen of interest, detectors include labeled antibodies or aptamers to a vesicle antigen of interest, and characterizing a phenotype includes a diagnosis, prognosis, or theranosis of a disease. In the scheme shown in **FIG. 2D i),** a population of vesicles is captured with one or more capture agents against general vesicle biomarkers **(6300).** The captured vesicles are then labeled with detectors against cell-of-origin biomarkers **(6301)** and/or disease specific biomarkers **(6302).** If only cell-of-origin detectors are used **(6301),** the biosignature used to characterize the phenotype **(6303)** can include the general vesicle markers **(6300)** and the cell-of-origin biomarkers **(6301).** If only disease detectors are

PREOMICS EXHIBIT 1002
Page 1071 of 1861

used **(6302),** the biosignature used to characterize the phenotype **(6303)** can include the general vesicle markers **(6300)** and the disease biomarkers **(6302).** Alternately, detectors are used to detect both cell-of-origin biomarkers **(6301)** and disease specific biomarkers **(6302).** In this case, the biosignature used to characterize the phenotype **(6303)** can include the general vesicle markers **(6300),** the cell-of-origin biomarkers **(6301)** and the disease biomarkers **(6302).** The biomarkers combinations are selected to characterize the phenotype of interest and can be selected from the biomarkers and phenotypes described herein.

[00497] In the scheme shown in **FIG. 2D ii),** a population of vesicles is captured with one or more capture agents against cell-of-origin biomarkers **(6310)** and/or disease biomarkers **(6311).** The captured vesicles are then detected using detectors against general vesicle biomarkers **(6312).** If only cell-of-origin capture agents are used **(6310),** the biosignature used to characterize the phenotype **(6313)** can include the cell-of-origin biomarkers **(6310)** and the general vesicle markers **(6312).** If only disease biomarker capture agents are used **(6311),** the biosignature used to characterize the phenotype **(6313)** can include the disease biomarkers **(6311)** and the general vesicle biomarkers **(6312).** Alternately, capture agents to one or more cell-of-origin biomarkers **(6310)** and one or more disease specific biomarkers **(6311)** are used to capture vesicles. In this case, the biosignature used to characterize the phenotype **(6313)** can include the cell-of-origin biomarkers **(6310),** the disease biomarkers **(6311),** and the general vesicle markers **(6313).** The biomarkers combinations are selected to characterize the phenotype of interest and can be selected from the biomarkers and phenotypes described herein.

[00498] Biomarkers comprising vesicle payload can be analyzed to characterize a phenotype. Payload comprises the biological entities contained within a vesicle membrane. These entities include without limitation nucleic acids, e.g., mRNA, microRNA, or DNA fragments; protein, e.g., soluble and membrane associated proteins; carbohydrates; lipids; metabolites; and various small molecules, e.g., hormones. The payload can be part of the cellular milieu that is encapsulated as a vesicle is formed in the cellular environment. In some embodiments of the invention, the payload is analyzed in addition to detecting vesicle surface antigens. Specific populations of vesicles can be captured as described above then the payload in the captured vesicles can be used to characterize a phenotype. For example, vesicles captured on a substrate can be further isolated to assess the payload therein. Alternately, the vesicles in a sample are detected and sorted without capture. The vesicles so detected can be further isolated to assess the payload therein. In an embodiment, vesicle populations are sorted by flow cytometry and the payload in the sorted vesicles is analyzed. In the scheme shown in **FIG. 2E iii),** a population of vesicles is captured and/or detected **(6320)** using one or more of cell-of-origin biomarkers **(6320),** disease biomarkers **(6321),** and general vesicle markers **(6322).** The vesicles can also be detected using one or more of angiogenic or immunomodulatory biomarkers.The payload of the isolated vesicles is assessed **(6323).** A biosignature detected within the payload can be used to characterize a phenotype **(6324).** In a non-limiting example, a vesicle population can be analyzed in a plasma sample from a patient using antibodies against one or more vesicle antigens of interest. The antibodies can be capture antibodies which are tethered to a substrate to isolate a desired vesicle population. Alternately, the antibodies can be directly labeled and the labeled vesicles isolated by sorting with flow cytometry. The presence or level of microRNA or mRNA extracted from the isolated vesicle population can be used to detect a biosignature. The biosignature is then used to diagnose, prognose or theranose the patient.

[00499] In other embodiments, vesicle payload is analyzed in a vesicle population without first capturing or detected subpopulations of vesicles. For example, vesicles can be generally isolated from a sample using

PREOMICS EXHIBIT 1002
Page 1072 of 1861

centrifugation, filtration, chromatography, or other techniques as described herein. The payload of the isolated vesicles can be analyzed thereafter to detect a biosignature and characterize a phenotype. In the scheme shown in **FIG. 2E iv),** a population of vesicles is isolated **(6330)** and the payload of the isolated vesicles is assessed **(6331).** A biosignature detected within the payload can be used to characterize a phenotype **(6332).** In a non-limiting example, a vesicle population is isolated from a plasma sample from a patient using size exclusion and membrane filtration. The presence or level of microRNA or mRNA extracted from the vesicle population is used to detect a biosignature. The biosignature is then used to diagnose, prognose or theranose the patient.

[00500] Another illustrative scheme for characterizing a phenotype is shown in **FIG. 2F v).** One or more vesicle of interest is captured and detected using a combination of cell-of-origin biomarkers **(6340)** and disease biomarkers **(6341).** For example, the vesicles of interest can be captured using a cell-of-origin **(6340)** biomarker and detected using a disease-specific **(6341)** biomarker. Similarly, the vesicles of interest can be captured using a disease-specific **(6341)** biomarker and detected using a cell-of-origin **(6340)** biomarker. If appropriate, the vesicle of interest can be captured and detected using only cell-of-origin **(6340)** biomarkers or only disease-specific **(6341)** biomarkers. In this case, the same biomarker could be used for capture and detection (e.g., anti-EpCAM capture and anti-EpCAM detector, or anti-PCSA capture and anti-PCSA detector, etc.), or different biomarkers from the same class can be used for capture and detection (e.g., anti-EpCAM capture and anti-B7H3 detector, or anti-PCSA capture and anti-PSMA detector, etc.). The phenotype can be characterized based on the detected vesicles. Optionally, payload **(6342)** in the vesicles of interest can be assessed in order to characterize the phenotype.

[00501] The methods of characterizing a phenotype can employ a combination of techniques to assess a vesicle population in a sample of interest. In an embodiment, the sample is split into various aliquots and each is analyzed separately. For example, protein content of one or more aliquot is determined and microRNA content of one or more other aliquot is determined. The protein content and microRNA content can be combined to characterize a phenotype. In another embodiment, vesicles of interest are isolated and the payload therein is assessed. For example, a population of vesicles with a given surface marker can be isolated by affinity isolation such as flow cytometry, immunoprecipitation, or other immunocapture technique using a binding agent to the surface marker of interest. The isolated vesicles can then be assessed for biomarkers such as surface content or payload. The biomarker profile of vesicles having the given surface marker can be used to characterize a phenotype. As a non-limiting example, a PCSA+ capture agent can be used to isolate a prostate specific vesicle population. Levels of surface antigens such as PCSA itself, PSMA, B7H3, or EpCam can be assessed from the PCSA+ vesicles. Levels of payload in the PCSA+ can also be assessed, e.g., microRNA or mRNA content. A biosignature can be constructed from a combination of the markers in the PCSA+ vesicle population.

[00502] In an embodiment, the invention provides a method of isolating a microvesicle population and assessing the microRNA with the isolated microvesicles. The microvesicle can be bound in a microtiter plate well that has been coated with a binding agent to a general vesicle biomarker, a cell-of-origin vesicle biomarker, or a disease-specific vesicle biomarker. As desired, vesicles in the wells can be detected using one or more detector agent to a general vesicle biomarker, a cell-of-origin vesicle biomarker, or a disease-specific vesicle biomarker. RNA can be isolated from microvesicles in wells that comprise the vesicles of interest. MicroRNA or miRNA content derived from the microvesicles are then detected. The presence or levels of the vesicle

PREOMICS EXHIBIT 1002
Page 1073 of 1861

markers and RNA markers can be used to construct a biosignature as described herein. The biosignature can be used to characterize a phenotype of interest.

[00503] In another embodiment, contaminants are removed from a biological sample and the remaining vesicles are assessed for surface content and/or payload. For example, a column can be constructed comprising binding agents to contaminating proteins, vesicles, or other entities in the biological sample. The flow through will thereby be enriched in the circulating biomarkers or circulating microvesicles of interest. In a non-limiting example, a column is constructed to remove microvesicles derived from blood cells. The column can be used to enrich microvesicles in a blood sample that are derived from non-blood cell origin. The enrichment scheme can be used to remove protein aggregates, nucleic acids in solution, etc. One of skill will appreciate that this enrichment can be used with other vesicle or biomarkers methodology presented herein to assess vesicle or biomarkers or interest. To continue the non-limiting example, the flow through that has been depleted in vesicles from blood cells can then be analyzed via a positive selection for vesicles of interest using affinity techniques or the like.

[00504] A peptide or protein biomarker can be analyzed by mass spectrometry or flow cytometry. Proteomic analysis of a vesicle may be carried out by immunocytochemical staining, Western blotting, electrophoresis, SDS-PAGE, chromatography, x-ray crystallography or other protein analysis techniques in accordance with procedures well known in the art. In other embodiments, the protein biosignature of a vesicle may be analyzed using 2 D differential gel electrophoresis as described in, *Chromy et al. J Proteome Res, 2004;3:1120-1127,* which is herein incorporated by reference in its entirety, or with liquid chromatography mass spectrometry as described in *Zhang et al. Mol Cell Proteomics, 2005;4:144-155,* which is herein incorporated by reference in its entirety. A vesicle may be subjected to activity-based protein profiling described for example, in *Berger et al, Am J Pharmacogenomics, 2004;4:371-381,* which is in incorporated by reference in its entirety. In other embodiments, a vesicle may be profiled using nanospray liquid chromatography-tandem mass spectrometry as described in *Pisitkun et al, Proc Natl Acad Sci USA, 2004; 101:13368-13373,* which is herein incorporated by reference in its entirety. In another embodiment, the vesicle may be profiled using tandem mass spectrometry (MS) such as liquid chromatography/MS/MS (LC-MS/MS) using for example a LTQ and LTQ-FT ion trap mass spectrometer. Protein identification can be determined and relative quantitation can be assessed by comparing spectral counts as described in *Smalley et al, J Proteome Res, 2008; 7.-2088-2096,* which is herein incorporated by reference in its entirety.

[00505] The expression of circulating protein biomarkers or protein payload within a vesicle can also be identified. The latter analysis can optionally follow the isolation of specific vesicles using capture agents to capture populations of interest. In an embodiment, immunocytochemical staining is used to analyze protein expression. The sample can be resuspended in buffer, centrifuged at 100 x g for example, for 3 minutes using a cytocentrifuge on adhesive slides in preparation for immunocytochemical staining. The cytospins can be air-dried overnight and stored at -80°C until staining. Slides can then be fixed and blocked with serum-free blocking reagent. The slides can then be incubated with a specific antibody to detect the expression of a protein of interest. In some embodiments, the vesicles are not purified, isolated or concentrated prior to protein expression analysis.

[00506] Biosignatures comprising vesicle payload can be characterized by analysis of a metabolite marker or metabolite within the vesicle. Various metabolite-oriented approaches have been described such as metabolite

PREOMICS EXHIBIT 1002
Page 1074 of 1861

target analyses, metabolite profiling, or metabolic fi¾ erprin¾ , see for example, *Denkert et al, Molecular Cancer 2008; 7: 4598-461 7, Ellis et al, Analyst 2006; 8: 875-885, Kuhn et al, Clinical Cancer Research 2007; 24: 7401-7406, Fiehn O., Comp Funct Genomics 2001;2:155-168, Fancy et al, Rapid Commun Mass Spectrom 20(15): 2271-80 (2006), Lindon et al, Pharm Res, 23(6): 1075-88 (2006), Holmes et al, Anal Chem. 2007 Apr 1; 79(7).-2629-40. Epub 2007 Feb 27. Erratum in: Anal Chem. 2008 Aug 1;80(15):6142-3, Stanley et al, Anal Biochem. 2005 Aug 15;343(2):195-202., Lehtimaki et al, J Biol Chem. 2003 Nov 14;278(46):45915-23,* each of which is herein incorporated by reference in its entirety.

[00507] Peptides can be analyzed by systems described in *Jain KK: Integrative Omics, Pharmacoproteomics, and Human Body Fluids. In: Thongboonkerd V, ed., ed. Proteomics of Human Body Fluids: Principles, Methods and Applications. Volume 1: Totowa, N.J. : Humana Press, 2007,* which is herein incorporated by reference in its entirety. This system can generate sensitive molecular fingerprints of proteins present in a body fluid as well as in vesicles. Commercial applications which include the use of chromatography/mass spectroscopy and reference libraries of all stable metabolites in the human body, for example Paradigm Genetic's Human Metabolome Project, may be used to determine a metabolite biosignature. Other methods for analyzing a metabolic profile can include methods and devices described in U.S. Patent No. 6,683,455 (Metabometrix), U.S. Patent Application Publication Nos. 20070003965 and 20070004044 (Biocrates Life Science), each of which is herein incorporated by reference in its entirety. Other proteomic profiling techniques are described in *Kennedy, Toxicol Lett 120:379-384 (2001), Berven et al, Curr Pharm Biotechnol 7(3): 147-58 (2006), Conrads et al, Expert Rev Proteomics 2(5): 693-703, Decramer et al, World J Urol 25(5): 457-65 (2007), Decramer et al, Mol Cell Proteomics 7(10): 1850-62 (2008), Decramer et al, Contrib Nephrol, 160: 127-41 (2008), Diamandis, JProteome Res 5(9): 2079-82 (2006), Immler et al, Proteomics 6(10): 2947-58 (2006), Khan et al, JProteome Res 5(10): 2824-38 (2006), Kumar et al, Biomarkers 11(5): 385-405 (2006), Noble et al, Breast Cancer Res Treat 104(2): 191-6 (2007), Omenn, Dis Markers 20(3): 131-4 (2004), Powell et al, Expert Rev Proteomics 3(1): 63-74 (2006), Rai et al, Arch Pathol Lab Med, 126(12): 1518-26 (2002), Ramstrom et al, Proteomics, 3(2): 184-90 (2003), Tammen et al, Breast Cancer Res Treat, 79(1): 83-93 (2003), Theodorescu et al, Lancet Oncol, 7(3): 230-40 (2006), or Zurbig et al, Electrophoresis, 27(11): 2111-25 (2006).*

[00508] For analysis of mRNAs, miRNAs or other small RNAs, the total RNA can be isolated using any known methods for isolating nucleic acids such as methods described in U.S. Patent Application Publication No. 2008132694, which is herein incorporated by reference in its entirety. These include, but are not limited to, kits for performing membrane based RNA purification, which are commercially available. Generally, kits are available for the small-scale (30 mg or less) preparation of RNA from cells and tissues, for the medium scale (250 mg tissue) preparation of RNA from cells and tissues, and for the large scale (1 g maximum) preparation of RNA from cells and tissues. Other commercially available kits for effective isolation of small RNA-containing total RNA are available. Such methods can be used to isolate nucleic acids from vesicles.

[00509] Alternatively, RNA can be isolated using the method described in U.S. Patent No. 7,267,950, which is herein incorporated by reference in its entirety. U.S. Patent No. 7,267,950 describes a method of extracting RNA from biological systems (cells, cell fragments, organelles, tissues, organs, or organisms) in which a solution containing RNA is contacted with a substrate to which RNA can bind and RNA is withdrawn from the substrate by applying negative pressure. Alternatively, RNA may be isolated using the method described in U.S. Patent Application No. 20050059024, which is herein incorporated by reference in its entirety, which describes

PREOMICS EXHIBIT 1002
Page 1075 of 1861

the isolation of small RNA molecules. Other methods are described in U.S. Patent Application No. 200502085 10, 20050277121, 200702381 18, each of which is incorporated by reference in its entirety.

[00510] In one embodiment, mRNA expression analysis can be carried out on mRNAs from a vesicle isolated from a sample. In some embodiments, the vesicle is a cell-of-origin specific vesicle. An expression pattern generated from a vesicle can be indicative of a given disease state, disease stage, therapy related signature, or physiological condition.

[00511] In one embodiment, once the total RNA has been isolated, cDNA can be synthesized and either qRT-PCR assays (e.g. Applied Biosystem's Taqman® assays) for specific mRNA targets can be performed according to manufacturer's protocol, or an expression microarray can be performed to look at highly multiplexed sets of expression markers in one experiment. Methods for establishing gene expression profiles include determining the amount of RNA that is produced by a gene that can code for a protein or peptide. This can be accomplished by quantitative reverse transcriptase PCR (qRT-PCR), competitive RT-PCR, real time RT-PCR, differential display RT-PCR, Northern Blot analysis or other related tests. While it is possible to conduct these techniques using individual PCR reactions, it is also possible to amplify complementary DNA (cDNA) or complementary RNA (cRNA) produced from mRNA and analyze it via microarray.

[00512] The level of a miRNA product in a sample can be measured using any appropriate technique that is suitable for detecting mRNA expression levels in a biological sample, including but not limited to Northern blot analysis, RT-PCR, qRT-PCR, in situ hybridization or microarray analysis. For example, using gene specific primers and target cDNA, qRT-PCR enables sensitive and quantitative miRNA measurements of either a small number of target miRNAs (via singleplex and multiplex analysis) or the platform can be adopted to conduct high throughput measurements using 96-well or 384-well plate formats. See for example, *Ross JS et al, Oncologist. 2008 May;13(5):477-93,* which is herein incorporated by reference in its entirety. A number of different array configurations and methods for microarray production are known to those of skill in the art and are described in U.S. patents such as: U.S. Pat. Nos. 5,445,934; 5,532,128; 5,556,752; 5,242,974; 5,384,261 ; 5,405,783; 5,412,087; 5,424,1 86; 5,429,807; 5,436,327; 5,472,672; 5,527,681 ; 5,529,756; 5,545,53 1; 5,554,501 ; 5,561,071 ; 5,571,639; 5,593,839; 5,599,695; 5,624,71 1; 5,658,734; or 5,700,637; each of which is herein incorporated by reference in its entirety. Other methods of profiling miRNAs are described in *Taylor et al, Gynecol Oncol. 2008 Jul;110(l):13-21, Gilad et al, PLoS ONE. 2008 Sep 5;3(9):e3148, Lee et al, Annu Rev Pathol. 2008 Sep 25 and Mitchell et al, Proc Natl Acad Sci USA. 2008 Jul 29;105(30):10513-8, Shen R et al, BMC Genomics. 2004 Dec 14;5(1):94, Mina L et al, Breast Cancer Res Treat. 2007 Jun; 103(2): 197-208, Zhang L et al, Proc Natl Acad Sci USA. 2008 May 13;105(19): 7004-9, Ross JS et al, Oncologist. 2008 May;13(5):477-93, Schetter AJ et al, JAMA. 2008 Jan 30;299(4):425-36, Staudt LM, N Engl J Med 2003;348:1 777-85, Mulligan G et al, Blood. 2007 Apr 15;109(8):3177-88. Epub 2006 Dec 21, McLendon R et al, Nature. 2008 Oct 23;455(7216):1061-8,* and U.S. Patent Nos. 5,538,848, 5,723,591, 5,876,930, 6,030,787, 6,258,569, and 5,804,375, each of which is herein incorporated by reference. In some embodiments, arrays of microRNA panels are use to simultaneously query the expression of multiple miRs. The Exiqon mIRCURY LNA microRNA PCR system panel (Exiqon, Inc., Woburn, MA) or the TaqMan® MicroRNA Assays and Arrays systems from Applied Biosystems (Foster City, CA) can be used for such purposes.

[00513] Microarray technology allows for the measurement of the steady-state mRNA or miRNA levels of thousands of transcripts or miRNAs simultaneously thereby presenting a powerful tool for identifying effects

PREOMICS EXHIBIT 1002
Page 1076 of 1861

such as the onset, arrest, or modulation of uncontrolled cell proliferation. Two microarray technologies, such as cDNA arrays and oligonucleotide arrays can be used. The product of these analyses are typically measurements of the intensity of the signal received from a labeled probe used to detect a cDNA sequence from the sample that hybridizes to a nucleic acid sequence at a known location on the microarray. Typically, the intensity of the signal is proportional to the quantity of cDNA, and thus mRNA or miRNA, expressed in the sample cells. A large number of such techniques are available and useful. Methods for determining gene expression can be found in U.S. Pat. No. 6,271,002 to Linsley, et al.; U.S. Pat. No. 6,21 8,122 to Friend, et al.; U.S. Pat. No. 6,21 8,1 14 to Peck et al.; or U.S. Pat. No. 6,004,755 to Wang, et al., each of which is herein incorporated by reference in its entirety.

[00514] Analysis of an expression level can be conducted by comparing such intensities. This can be performed by generating a ratio matrix of the expression intensities of genes in a test sample versus those in a control sample. The control sample may be used as a reference, and different references to account for age, ethnicity and sex may be used. Different references can be used for different conditions or diseases, as well as different stages of diseases or conditions, as well as for determining therapeutic efficacy.

[00515] For instance, the gene expression intensities of mRNA or miRNAs derived from a diseased tissue, including those isolated from vesicles, can be compared with the expression intensities of the same entities in normal tissue of the same type (e.g., diseased breast tissue sample versus normal breast tissue sample). A ratio of these expression intensities indicates the fold-change in gene expression between the test and control samples. Alternatively, if vesicles are not normally present in from normal tissues (e.g. breast) then absolute quantitation methods, as is known in the art, can be used to define the number of miRNA molecules present without the requirement of miRNA or mRNA isolated from vesicles derived from normal tissue.

[00516] Gene expression profiles can also be displayed in a number of ways. A common method is to arrange raw fluorescence intensities or ratio matrix into a graphical dendogram where columns indicate test samples and rows indicate genes. The data is arranged so genes that have similar expression profiles are proximal to each other. The expression ratio for each gene is visualized as a color. For example, a ratio less than one (indicating down-regulation) may appear in the blue portion of the spectrum while a ratio greater than one (indicating up-regulation) may appear as a color in the red portion of the spectrum. Commercially available computer software programs are available to display such data.

[00517] mRNAs or miRNAs that are considered differentially expressed can be either over expressed or under expressed in patients with a disease relative to disease free individuals. Over and under expression are relative terms meaning that a detectable difference (beyond the contribution of noise in the system used to measure it) is found in the amount of expression of the mRNAs or miRNAs relative to some baseline. In this case, the baseline is the measured mRNA/miRNA expression of a non-diseased individual. The mRNA/miRNA of interest in the diseased cells can then be either over or under expressed relative to the baseline level using the same measurement method. Diseased, in this context, refers to an alteration of the state of a body that interrupts or disturbs, or has the potential to disturb, proper performance of bodily functions as occurs with the uncontrolled proliferation of cells. Someone is diagnosed with a disease when some aspect of that person's genotype or phenotype is consistent with the presence of the disease. However, the act of conducting a diagnosis or prognosis includes the determination of disease/status issues such as determining the likelihood of relapse or metastasis and therapy monitoring. In therapy monitoring, clinical judgments are made regarding the effect of a

PREOMICS EXHIBIT 1002
Page 1077 of 1861

given course of therapy by comparing the expression of genes over time to determine whether the mRNA/miRNA expression profiles have changed or are changing to patterns more consistent with normal tissue.

[00518] Levels of over and under expression are distinguished based on fold changes of the intensity measurements of hybridized microarray probes. A 2X difference is preferred for making such distinctions or a p-value less than 0.05. That is, before an mRNA/miRNA is the to be differentially expressed in diseased/relapsing versus normal/non-relapsing cells, the diseased cell is found to yield at least 2 times more, or 2 times less intensity than the normal cells. The greater the fold difference, the more preferred is use of the gene as a diagnostic or prognostic tool. mRNA/miRNAs selected for the expression profiles of the instant invention have expression levels that result in the generation of a signal that is distinguishable from those of the normal or non-modulated genes by an amount that exceeds background using clinical laboratory instrumentation.

[00519] Statistical values can be used to confidently distinguish modulated from non-modulated mRNA/miRNA and noise. Statistical tests find the mRNA/miRNA most significantly different between diverse groups of samples. The Student's t-test is an example of a robust statistical test that can be used to find significant differences between two groups. The lower the p-value, the more compelling the evidence that the gene shows a difference between the different groups. Nevertheless, since microarrays measure more than one mRNA/miRNA at a time, tens of thousands of statistical tests may be performed at one time. Because of this, one is unlikely to see small p-values just by chance and adjustments for this using a Sidak correction as well as a randomization/permutation experiment can be made. A p-value less than 0.05 by the t-test is evidence that the gene is significantly different. More compelling evidence is a p-value less then 0.05 after the Sidak correction is factored in. For a large number of samples in each group, a p-value less than 0.05 after the randomization/permutation test is the most compelling evidence of a significant difference.

[00520] In one embodiment, a method of generating a posterior probability score to enable diagnostic, prognostic, therapy-related, or physiological state specific biosignature scores can be arrived at by obtaining circulating biomarker expression data from a statistically significant number of patients; applying linear discrimination analysis to the data to obtain selected biomarkers; and applying weighted expression levels to the selected biomarkers with discriminate function factor to obtain a prediction model that can be applied as a posterior probability score. Other analytical tools can also be used to answer the same question such as, logistic regression and neural network approaches.

[00521] For instance, the following can be used for linear discriminant analysis:

where,

$I(p_s i_d)$ = The log base 2 intensity of the probe set enclosed in parenthesis. d(cp) = The discriminant function for the disease positive class $\mathbf{CI(CN)}$ = The discriminant function for the disease negative class

$\mathbf{P}(cp)$ = The posterior p-value for the disease positive class

$\mathbf{P(CN)}$ = The posterior p-value for the disease negative class

[00522] Numerous other well-known methods of pattern recognition are available. The following references provide some examples: *Weighted Voting: Golub et al. (1999); Support Vector Machines: Su et al. (2001); and Ramaswamy et al. (2001); K-nearest Neighbors : Ramaswamy (2001); and Correlation Coefficients: van 't Veer et al. (2002),* all of which are herein incorporated by reference in their entireties.

PREOMICS EXHIBIT 1002
Page 1078 of 1861

[00523] A biosignature portfolio, further described below, can be established such that the combination of biomarkers in the portfolio exhibit improved sensitivity and specificity relative to individual biomarkers or randomly selected combinations of biomarkers. In one embodiment, the sensitivity of the biosignature portfolio can be reflected in the fold differences, for example, exhibited by a transcript's expression in the diseased state relative to the normal state. Specificity can be reflected in statistical measurements of the correlation of the signaling of transcript expression with the condition of interest. For example, standard deviation can be a used as such a measurement. In considering a group of biomarkers for inclusion in a biosignature portfolio, a small standard deviation in expression measurements correlates with greater specificity. Other measurements of variation such as correlation coefficients can also be used in this capacity.

[00524] Another parameter that can be used to select mRNA/miRNA that generate a signal that is greater than that of the non-modulated mRNA/miRNA or noise is the use of a measurement of absolute signal difference. The signal generated by the modulated mRNA/miRNA expression is at least 20% different than those of the normal or non-modulated gene (on an absolute basis). It is even more preferred that such mRNA/miRNA produce expression patterns that are at least 30% different than those of normal or non-modulated mRNA/miRNA.

[00525] MiRNA can also be detected and measured by amplification from a biological sample and measured using methods described in U.S. Patent No. 7,250,496, U.S. Application Publication Nos. 20070292878, 20070042380 or 20050222399 and references cited therein, each of which is herein incorporated by reference in its entirety. The microRNA can be assessed as in U.S. Patent No. 7,888,035, entitled "METHODS FOR ASSESSING RNA PATTERNS," issued February 15, 2011, which application is incorporated by reference herein in its entirety.

[00526] The levels of microRNA can be normalized using various techniques known to those of skill in the art. For example, relative quantification of miRNA expression can be performed using the $2^{-\Delta\Delta C_T}$ method (Applied Biosystems User Bulletin N°2). The levels of microRNA can also be normalized to housekeeping nucleic acids, such as housekeeping mRNAs, microRNA or snoRNA. Further methods for normalizing miRNA levels that can be used with the invention are described further in Vasilescu, MicroRNA fingerprints identify miR-150 as a plasma prognostic marker in patients with sepsis. PLoS One. 2009 Oct 12;4(10):e7405; and Peltier and Latham, Normalization of microRNA expression levels in quantitative RT-PCR assays: identification of suitable reference RNA targets in normal and cancerous human solid tissues. RNA. 2008 May;14(5):844-52. Epub 2008 Mar 28; each of which reference is herein incorporated by reference in its entirety.

[00527] Peptide nucleic acids (PNAs) which are a new class of synthetic nucleic acid analogs in which the phosphate-sugar polynucleotide backbone is replaced by a flexible pseudo-peptide polymer may be used in analysis of a biosignature. PNAs are capable of hybridizing with high affinity and specificity to complementary RNA and DNA sequences and are highly resistant to degradation by nucleases and proteinases. Peptide nucleic acids (PNAs) are an attractive new class of probes with applications in cytogenetics for the rapid in situ identification of human chromosomes and the detection of copy number variation (CNV). Multicolor peptide nucleic acid-fluorescence in situ hybridization (PNA-FISH) protocols have been described for the identification of several human CNV-related disorders and infectious diseases. PNAs can also be used as molecular diagnostic tools to non-invasively measure oncogene mRNAs with tumor targeted radionuclide-PNA-peptide chimeras. Methods of using PNAs are described further in *Pellestor F et al, Curr Pharm Des. 2008;14(24):2439-44, Tian*

PREOMICS EXHIBIT 1002
Page 1079 of 1861

X et al, Ann N YAcad Sci. 2005 Nov; 1059: 106-44, Paulasova P and Pellestor F, Annales de Genetique, 47 (2004) 349-358, Stender H. Expert Rev Mol Diagn. 2003 Sep;3(5):649-55. Review, Vigneault et al, Nature Methods, 5(9), 777 - 779 (2008), each reference is herein incorporated by reference in its entirety. These methods can be used to screen the genetic materials isolated from a vesicle. When applying these techniques to a cell-of-origin specific vesicle, they can be used to identify a given molecular signal that directly pertains to the cell of origin.

[00528] Mutational analysis may be carried out for mRNAs and DNA, including those that are identified from a vesicle. For mutational analysis of a target or biomarker that is of RNA origin, the RNA (mRNA, miRNA or other) can be reverse transcribed into cDNA and subsequently sequenced or assayed, such as for known SNPs (by Taqman SNP assays, for example) or single nucleotide mutations, as well as using sequencing to look for insertions or deletions to determine mutations present in the cell-of-origin. Multiplexed ligation dependent probe amplification (MLPA) could alternatively be used for the purpose of identifying CNV in small and specific areas of interest. For example, once the total RNA has been obtained from isolated colon cancer-specific vesicles, cDNA can be synthesized and primers specific for exons 2 and 3 of the KRAS gene can be used to amplify these two exons containing codons 12, 13 and 61 of the KRAS gene. The same primers used for PCR amplification can be used for Big Dye Terminator sequence analysis on the ABI 3730 to identify mutations in exons 2 and 3 of KRAS. Mutations in these codons are known to confer resistance to drugs such as Cetuximab and Panitumimab. Methods of conducting mutational analysis are described in *Maheswaran S et al, July 2, 2008 (10.1056/NEJMoa0800668) and Orita, Met al, PNAS 1989, (86): 2766-70,* each of which is herein incorporated by reference in its entirety.

[00529] Other methods of conducting mutational analysis include miRNA sequencing. Applications for identifying and profiling miRNAs can be done by cloning techniques and the use of capillary DNA sequencing or "next-generation" sequencing technologies. The new sequencing technologies currently available allow the identification of low-abundance miRNAs or those exhibiting modest expression differences between samples, which may not be detected by hybridization-based methods. Such new sequencing technologies include the massively parallel signature sequencing (MPSS) methodology described in *Nakano et al. 2006, Nucleic Acids Res. 2006;34:D731-D735. doi: 10.1093/nar/gkj077,* the Roche/454 platform described in *Margulies et al. 2005, Nature. 2005;437:376-380* or the Illumina sequencing platform described in *Berezikov et al. Nat. Genet. 2006b;38: 1375-1377,* each of which is incorporated by reference in its entirety.

[00530] Additional methods to determine a biosignature includes assaying a biomarker by allele-specific PCR, which includes specific primers to amplify and discriminate between two alleles of a gene simultaneously, single-strand conformation polymorphism (SSCP), which involves the electrophoretic separation of single-stranded nucleic acids based on subtle differences in sequence, and DNA and RNA aptamers. DNA and RNA aptamers are short oligonucleotide sequences that can be selected from random pools based on their ability to bind a particular molecule with high affinity. Methods of using aptamers are described in Ulrich H et al, Comb Chem High Throughput Screen. 2006 Sep;9(8):619-32, Ferreira CS et al, Anal Bioanal Chem. 2008 Feb;390(4): 1039-50, Ferreira CS et al, Tumour Biol. 2006;27(6):289-301, each of which is herein incorporated by reference in its entirety.

[00531] Biomarkers can also be detected using fluorescence in situ hybridization (FISH). Methods of using FISH to detect and localize specific DNA sequences, localize specific mRNAs within tissue samples or identify

PREOMICS EXHIBIT 1002
Page 1080 of 1861

chromosomal abnormalities are described in *Shaffer DR et al, Clin Cancer Res. 2007 Apr 1;13(7):2023-9, Cappuzo F et al, Journal of Thoracic Oncology, Volume 2, Number 5, May 2007, Moroni M et al, Lancet Oncol. 2005 May;6(5):279-86,* each of which is herein incorporated by reference in its entirety.

[00532] An illustrative schematic for analyzing a population of vesicles for their payload is presented in **FIG. 2E.** In an embodiment, the methods of the invention include characterizing a phenotype by capturing vesicles **(6330)** and determining a level of microRNA species contained therein **(6331),** thereby characterizing the phenotype **(6332).**

[00533] A biosignature comprising a circulating biomarker or vesicle can comprise a binding agent thereto. The binding agent can be a DNA, RNA, aptamer, monoclonal antibody, polyclonal antibody, Fabs, Fab', single chain antibody, synthetic antibody, aptamer (DNA/RNA), peptoid, zDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), lectin, synthetic or naturally occurring chemical compounds (including but not limited to drugs and labeling reagents).

[00534] A binding agent can used to isolate or detect a vesicle by binding to a component of the vesicle, as described above. The binding agent can be used to detect a vesicle, such as for detecting a cell-of-origin specific vesicle. A binding agent or multiple binding agents can themselves form a binding agent profile that provides a biosignature for a vesicle. For example, if a vesicle population is detected or isolated using two, three, four or more binding agents in a differential detection or isolation of a vesicle from a heterogeneous population of vesicles, the particular binding agent profile for the vesicle population provides a biosignature for the particular vesicle population.

[00535] As an illustrative example, a vesicle for characterizing a cancer can be detected with one or more binding agents including, but not limited to, PSA, PSMA, PCSA, PSCA, B7H3, EpCam, TMPRSS2, mAB 5D4, XPSM-A9, XPSM-A10, Galectin-3, E-selectin, Galectin-1, or E4 (IgG2a kappa), or any combination thereof.

[00536] The binding agent can also be for a general vesicle biomarker, such as a "housekeeping protein" or antigen. The biomarker can be CD9, CD63, or CD81 . For example, the binding agent can be an antibody for CD9, CD63, or CD81 . The binding agent can also be for other proteins, such as for tissue specific or cancer specific vesicles. The binding agent can be for PCSA, PSMA, EpCam, B7H3, or STEAP. The binding agent can be for DR3, STEAP, epha2, TMEM21 1, MFG-E8, Annexin V, TF, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, or TETS. For example, the binding agent can be an antibody or aptamer for PCSA, PSMA, EpCam, B7H3, DR3, STEAP, epha2, TMEM21 1, MFG-E8, Annexin V, TF, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, or TETS.

[00537] Various proteins are not typically distributed evenly or uniformly on a vesicle shell. Vesicle-specific proteins are typically more common, while cancer-specific proteins are less common. In some embodiments, capture of a vesicle is accomplished using a more common, less cancer-specific protein, such as one or more housekeeping proteins or antigen or general vesicle antigen (e.g., a tetraspanin), and one or more cancer-specific biomarkers and/or one or more cell-of-origin specific biomarkers is used in the detection phase. In another embodiment, one or more cancer-specific biomarkers and/or one or more cell-of-origin specific biomarkers are used for capture, and one or more housekeeping proteins or antigen or general vesicle antigen (e.g., a tetraspanin) is used for detection. In embodiments, the same biomarker is used for both capture and detection. Different binding agents for the same biomarker can be used, such as antibodies or aptamers that bind different epitopes of an antigen.

PREOMICS EXHIBIT 1002
Page 1081 of 1861

[00538] Additional cellular binding partners or binding agents may be identified by any conventional methods known in the art, or as described herein, and may additionally be used as a diagnostic, prognostic or therapy-related marker. For example, vesicles can be detected using one or more binding agent listed in **Tables 3, 4** or **5** herein. For example, the binding agent can also be for a general vesicle biomarker, such as a "housekeeping protein" or antigen. The general vesicle biomarker can be CD9, CD63, or CD81, or other biomarker in **Table 3.** The binding agent can also be for other proteins, such as for cell of origin specific or cancer specific vesicles. As a non-limiting example, in the case of prostate cancer, the binding agent can be for PCSA, PSMA, EpCam, B7H3, RAGE or STEAP. The binding agent can be for a biomarker in **Tables 4-5.** For example, the binding agent can be an antibody or aptamer for PCSA, PSMA, EpCam, B7H3, RAGE, STEAP or other biomarker in **Tables 4-5.**

[00539] Various proteins may not be distributed evenly or uniformly on a vesicle surface. For example, vesicle-specific proteins are typically more common, while cancer-specific proteins are less common. In some embodiments, capture of a vesicle is accomplished using a more common, less cancer-specific protein, such as a housekeeping protein or antigen, and cancer-specific proteins is used in the detection phase. Depending on the sensitivity of the detection system, the opposite method can also be used wherein a large vesicle population is captured using a binding agent to a general vesicle marker and then cell-specific vesicles are detected with detection agents specific to a sub-population of interest.

[00540] Furthermore, additional cellular binding partners or binding agents may be identified by any conventional methods known in the art, or as described herein, and may additionally be used as a diagnostic, prognostic or therapy-related marker.

microRNA Functional Assay

[00541] As described above, microRNAs can be found circulating in bodily fluids such as blood encapsulated in microvesicles, HDL and LDL particles as well as components of ribonucleoprotein complexes (RNPs). microRNA can be detected using available technologies such as described herein or known in the art, including without limitation RT-qPCR or next generation sequencing. However, microRNA in a biologically active state is bound and activated by one or more of the Argonaute ("Ago") proteins (e.g., Agol, Ago2, Ago3, or Ago4). One aspect of the invention is directed to compositions and methods that enable detection of a functional activity of a target microRNA within a biological sample in a single reaction. For a review of the Ago family of proteins, *see,* Hock and Meister, Genome Biology, 2008, **9**:210.

[00542] More particularly, a substrate, a synthetic RNA molecule, a label and RISC (RNA-Induced Silencing Complex) reaction buffer components, and optionally one or more isolated Ago protein, are used to assess one or more nucleic acid biomarkers (e.g., microRNAs). Examples of a substrate that can be used in the invention include but are not limited to a planar substrate, microbead, column or the like to which a first section of a synthetic RNA molecule, e.g., the 3' or 5' end, is tethered via direct or indirect linkage. Such substrates are disclosed herein or known in the art. The linkage is performed using methods known in the art, e.g., amino-carboxy coupling such as described in Wittebolle et al., Optimisation of the amino-carboxy coupling of oligonucleotides to beads used in liquid arrays, J Chem Tech Biotech **81**:476-480 (2006); such techniques are readily known to a person having ordinary skill in the art.

[00543] Another portion of other the synthetic RNA molecule, e.g., the opposing 3' or 5' end, is attached directly or indirectly to a label or detectable molecule. The label is any molecule that is capable of being

PREOMICS EXHIBIT 1002
Page 1082 of 1861

detected, and such labels or detectable molecules are known in the art and include without limitation: a fluorescent label, radiolabel or enzymatic label. Additional examples of such labels are disclosed herein above. In between the substrate-tethered portion and the labeled portion, the synthetic RNA molecule comprises a section or portion that is complementary to a target microRNA of interest. As desired, the complementary section can be perfectly complementary to the target microRNA, i.e., 100% complementary. The degree of association between the complementary section and the target microRNA can be manipulated, e.g., to allow the recognition of one specific target microRNA or to allow promiscuous recognition, e.g., of a family of target microRNAs. Means for such manipulation are disclosed herein or are known in the art, e.g., base pair mismatches, or assay conditions such as temperature or salt concentration. For example, the complementary section may carry mismatches with the target microRNA, e.g., such that the complementary section is at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99% complementary to the target microRNA. The method comprises contacting the labeled and tethered synthetic RNA molecule with a sample comprising or suspected to comprise the target microRNA of interest. If the target microRNA is present in the sample and is also bound to an Ago protein, the Ago-microRNA can associate with the synthetic RNA molecule via base pairing between the target microRNA and the complementary region. Such association facilitates the cleavage of the synthetic RNA molecule via the endonucleolytic cleavage activity of the Ago protein. This cleavage liberates the label of the synthetic RNA molecule from the substrate. The amount of label associated with the substrate can be detected before and after contact with the sample comprising the target microRNA. Any such differences in the amount of label are indicative of the amount of Ago-bound target microRNA in the input sample.

[00544] Useful reaction conditions and buffers for the assay are known in the art. The reaction be performed at room temperature, 25°C, 30°C, 37°C or up to 42°C-45°C for anywhere from 5 min to overnight depending on assay sensitivity and target abundance. For example, the reaction can be performed for 1-2 h at 37°C. *See, e.g.,* Brown et al., Target accessibility dictates the potency of human RISC. *Nature Structural & Molecular Biology* **12,** 469 - 470 (2005); Robb et al., Specific and potent RNAi in the nucleus of human cells. Nature Structural & Molecular Biology 12, 133 - 137 (2005); Lima et al., Binding and Cleavage Specificities of Human Argonaute2. J. Biol. Chem. 2009 284: 26017-26028.

[00545] An exemplary embodiment of the assay is shown in **FIG. 26.** As shown in **FIG. 26A,** a synthetic RNA molecule contains a 3' linker/extender region **262,** a central miRNA targeting region **263** and a second 5'linker/extension region **264.** The RNA is attached to a substrate, here microbead **261,** on the 3'end **262** and the 5'end **264** is conjugated with biotin **266.** The central miRNA targeting region **263** is designed to complement a miRNA sequence of interest. Region **263** can be complementary to any microRNA of interest. In the example shown in **FIG. 26,** streptavidin-PE (Phycoerythrin) **265** is used to label the biotin end of the synthetic RNA. As described, other labeling schemes can be employed. For example, the 5'end **264** can be directly labeled with Cy3, Cy5 or other detectable moiety disclosed herein or known in the art. As another example, the 5'end **264** can be indirectly labeled via base pairing with another complementary oligonucleotide that is labeled. If the target microRNA is present in the sample and is bound/associated with an Ago protein **267,** e.g., any of Ago 1-4 in the sample or added thereto, such as recombinant Ago2 (rAgo2), the target microRNA will bind the complementary microRNA targeting region **263** and subsequently cleave the synthetic RNA at region **263** through the endonucleolytic cleavage activity of Argonaute. *See* step **268** in **FIG. 26.** Once cleaved, the labeled

PREOMICS EXHIBIT 1002
Page 1083 of 1861

end (here 5') of the synthetic RNA molecule is released, thereby separating the biotin/Streptavidin-PE complex **265-266** from the microbead **261.** See **FIG. 26B.** Next, the substrate microbeads can be isolated and washed to remove the cleaved and untethered end of the RNA, thereby leaving only the remaining uncleaved and still labeled material as well as any cleaved but now unlabeled RNA. After this wash step, the difference in PE signal correlates with the concentration and activity of the Ago-bound target microRNA **267** present in the original assay. The quantity of Ago-bound target microRNA in the input sample determines the level of RNA cleaved. For example, if the target microRNA is not present, or it is present but not bound in a functional form with Ago, the synthetic RNA target region **263** will remain uncleaved and the signal strength will be unchanged.

[00546] Any appropriate source of RNA and/or RNA pre-loaded into Argonaute can be tested using the assay. For example, the input sample may be cell lysate, bodily fluids, blood fractions (which may contain circulating Argonaute such as Ago 2 bound to miRNAs), plasma, serum, or isolated microvesicles. In some embodiments, Argonaute immunoprecipitated from a sample is used as an input source of RNP complexes for the assay. If the target microRNA is present and loaded into Argonaute in any of the aforementioned sources, the synthetic target **263** is cleaved and the label (e.g., biotin-strepavidin-PE **265-266** in the example of **FIG. 26)** is released.

[00547] **FIGs. 26C-E** illustrate schematically various sources of RNA that can be used as input for the assay. **FIG. 26C** illustrates microRNA **268** bound to an Ago protein **269** to form a ribonucleic acid complex **267.** The Ago protein can be Agol, Ago 2, Ago3 or Ago 4. **FIG. 26D** illustrates immunoprecipitation of an Argonaute - microRNA complex **267** using a binding agent to Ago **2610.** The binding agent can be specific to a certain Argonaute, e.g., an antibody or aptamer to Ago2. In other embodiments, the binding agent recognizes more than one Ago family member, e.g., Agol-4. In still other embodiments, the binding agent can bind indirectly to the one or more Ago protein. For example, the binding agent for the immunoprecipitation can be an antibody or aptamer to GW1 82 protein which forms a complex with Ago proteins. **FIG. 26E** illustrates direct analysis of Argonaute - microRNA complex **267,** e.g., from a cell lysate, bodily fluid, or lysed microvesicle.

[00548] Alternately, the assay input can comprise RNA from a sample source bound that is then contacted with an Ago protein, such as purified Ago including recombinant Ago (rAgo). In this manner, RNA can be isolated from any appropriate source including without limitation cell lysate, bodily fluids, plasma, concentrated plasma, microvesicles, or HDL and LDL particles. Once isolated, the Ago protein, e.g., recombinant Argonaute 2, can be used to bind small RNA present in the sample. The Ago bound RNA can be used as input into the assay.

[00549] As described above, the third portion of the synthetic RNA molecule is labeled and thus cleavage of the complementary section allows removal of the label from the substrate. Thus, the amount of label removed from the substrate corresponds to the number of cleavage events. It will be appreciated that alternate methods of detecting the cleavage events are within the scope of the invention. In one embodiment, the label is added to the reaction mixture after the cleavage reaction has been allowed to occur. Following the example above, the streptavidin-PE **265** is added after the cleavage reaction has taken place. In another example, the third portion of the synthetic RNA molecule is not labeled. Rather, the cleavage events are observed by detecting the amount of cleaved synthetic RNA molecule remaining on the column after the cleavage reaction has occurred.

[00550] The degree of label liberated from the substrate can be detected and compared before and after the cleavage reaction has taken place. Alternately, the kinetics of the cleavage reaction can be observed using the subject methods. In an embodiment, the degree of label liberated from the substrate is detected in real time, thereby revealing the kinetics of the cleavage reaction.

PREOMICS EXHIBIT 1002
Page 1084 of 1861

[00551] Using the microRNA functional assay, virtually any microRNA can be screened with synthetic RNAs containing matched miRNA targeting regions. The assay can be performed in uniplex or multiplex fashion with multiple synthetic targets attached to distinguishable microbeads.

[00552] In an embodiment, the miR assay system is used for therapeutic RNAi molecule delivery and mode of action confirmation. Here, RNAi molecules are delivered systemically or in a targeted fashion to an appropriate cell type, tissue or other anatomical region. Target tissues can be analyzed for confirmation of delivery and confirmation of the RNAi therapeutic mode of action. For example, the presence of a therapeutic RNAi molecule at the tissue of interest can be detected by a phenotypic result directly driven by mRNA knockdown due to the activation of the RNAi therapeutic or alternatively through an unrelated apoptotic or inflammatory response of the cell. Lastly, IC50 of the activated therapeutic RNAi agent at the target tissue can be established using this methodology.

Biosignatures for Cancer

[00553] As described herein, biosignatures comprising circulating biomarkers can be used to characterize a cancer. The biomarkers can be selected from those disclosed herein. For example, a non-exclusive list of biomarkers that can be used as part of a biosignature are listed in **Table 5.** The biosignature can be used to characterize a cancer, e.g., for prostate, GI, or ovarian cancer. In some embodiments, the circulating biomarkers are associated with a vesicle or with a population of vesicles. For example, circulating biomarkers associated with vesicles can be used to capture and/or to detect a vesicle or a vesicle population.

[00554] It will be appreciated that the biomarkers presented herein, e.g., in **Table 5,** may be useful in biosignatures for other diseases, e.g., other proliferative disorders and cancers of other cellular or tissue origins. For example, transformation in various cell types can be due to common events, e.g., mutation in p53 or other tumor suppressor. A biosignature comprising cell-of-origin biomarkers and cancer biomarkers can be used to further assess the nature of the cancer. Biomarkers for metastatic cancer may be used with cell-of-origin biomarkers to assess a metastatic cancer. Such biomarkers for use with the invention include those in Dawood, Novel biomarkers of metastatic cancer, Exp Rev Mol Diag July 2010, Vol. 10, No. 5, Pages 581-590, which publication is incorporated herein by reference in its entirety.

[00555] For example, a biosignature comprising one or more of miR-378, miR-127-3p, miR-92a, and miR-486-3p can be used to characterize colorectal cancer. The presence of KRAS mutations can be associated with miR expression levels. See, e.g., Mosakhani et al., MicroRNA profiling differentiates colorectal cancer according to KRAS status. Genes Chromosomes Cancer. 201 1 Sep 15. doi: 10.1002/gcc.20925, which publication is incorporated herein by reference in its entirety. For example, KRAS mutations can be associated with up-regulation miR-127-3p, miR-92a, and miR-486-3p and down-regulation of miR-378. Somatic KRAS mutations are found at high rates in various disorders, including without limitation leukemias, colon cancer, pancreatic cancer and lung cancer. KRAS mutations are predictive of poor response to panitumumab and cetuximab therapy. A KRAS+ phenotype is also associated with poor response to anti-EGFR therapies such as erlotinib and/or gefitinib. Thus, in an embodiment, levels of miRs correlated with KRAS status are used as part of a biosignature to provide a theranosis for cancers, e.g., metastatic colorectal cancer or lung cancer.

[00556] As another example, Pgrmcl can be elevated in lung cancer tissue compared to normal tissue and in the plasma of lung cancer patients compared to non-cancer patients. See, e.g., Mir et al., Elevated Pgrmcl (progesterone receptor membrane component 1)/sigma-2 receptor levels in lung tumors and plasma from lung

PREOMICS EXHIBIT 1002
Page 1085 of 1861

cancer patients. Int J Cancer. 201 1 Sep 14. doi: 10. 1002/ijc.26432, which publication is incorporated herein by reference in its entirety. In an embodiment, a presense or level of circulating Pgrmcl is assessed in a patient sample in order to characterize a cancer. The cancer can be a lung cancer, including without limitation a squamous cell lung cancer (SCLC) or a lung adenocarcinoma. Elevated levels of Pgrmcl compared to a control can indicate the presense of the cancer. The sample can be a tissue sample or a bodily fluid, e.g, sputum, peripheral blood, or a blood derivative. In an embodiment, the Pgrmcl is associated with a population of vesicles.

[00557] The biosignatures of the invention may comprise markers that are upregulated, downregulated, or have no change, depending on the reference. Solely for illustration, if the reference is a normal sample, the biosignature may indicate that the subject is normal if the subject's biosignature is not changed compared to the reference. Alternately, the biosignature may comprise a mutated nucleic acid or amino acid sequence so that the levels of the components in the biosignature are the same between a normal reference and a diseased sample. In another case, the reference can be a cancer sample, such that the subject's biosignature indicates cancer if the subject's biosignature is substantially similar to the reference. The biosignature of the subject can comprise components that are both upregulated and downregulated compared to the reference. Solely for illustration, if the reference is a normal sample, a cancer biosignature can comprise both upregulated oncogenes and downregulated tumor suppressors. Vesicle markers can also be differentially expressed in various settings. For example, tetraspanins may be overexpressed in cancer vesicles compared to non-cancer vesicles, whereas MFG-E8 can be overexpressed in non-cancer vesicles as compared to cancer vesicles.

[00558] ***Prostate Cancer Biosignatures***

[00559] In an aspect, the invention provides a method of detecting a microvesicle population in a biological sample. In an embodiment, the method comprises detecting a biosignature comprising a presence or level of multiple biomarkers. The biosignature can be used to characterize a cancer, e.g., a prostate cancer.

[00560] In an embodiment, the method comprises: (a) contacting a microvesicle population in a biological sample with a first binding agent and a second binding agent, (b) determining a presence or level of the microvesicle population bound by the first and second binding agents; and (c) identifying a biosignature comprising the presence or level of the bound microvesicle population. The first and second binding agents can comprise a pair of binding agents. The pair of binding agents can be used to identify a microvesicle population using various methods disclosed herein or known in the art. For example, the pair can be used to label a pair of antigens on a microvesicle surface. The labeled microvesicle population can be detected using flow cytometry or the like. Alternately, one member of the pair can be bound to a substrate (e.g., a capture agent) and the other member can be used to label the microvesicle, wherein the label allows detection of microvesicles bound by the pair of binding agents. The substrate can be a well, array, bead, column, paper, or the like as described herein or known in the art. The label can be a fluorescent, radiolabeled, enzymatic, or the like as described herein or known in the art. The label can also be indirect. For example, the labeled member of the binding pair may comprise a biotin molecule to allow its labeling with an avidin-bound label. Similarly, the labeled member of the binding pair can be detected by another labeled binding agent, e.g., a mouse IgG antibody binding agent can be labeled with a directly labeled anti-mouse IgG antibody. Any such configurations are contemplated by the invention.

PREOMICS EXHIBIT 1002
Page 1086 of 1861

[00561] In an embodiment, the first binding agent comprises a capture agent and the second binding agent comprises a detector agent. The capture and detector agents can be selected from one or more, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, 25, or all, pair of capture and detector agents in any of **Tables 38, 40-44, 50, 51, 55-67,** and **72-74.** For example, the capture and detector agents can be selected from one or more, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, 25, or all, pair of capture and detector agents in any of **Tables 38, 50, 55** and **72.** Multiple pairs of capture and detector agents may improve the ability to characterize a phenotype. Thus, the invention contemplates use of any pairs of capture and detector agents that provide the desired diagnostic, prognostic or theranostic readout. As described herein, the use of the capture/detector pairs allows detection of microvesicle populations carrying more than one biomarker, e.g., one marker can be a tissue-specific or cell-of-origin marker, and the other marker can be a cancer marker. This scenario would allow detection of microvesicles that are shed from cancer cells from a given anatomical tissue or location. Thus, one of skill will appreciate that the targets of the capture/detector pairs can be switched while still detecting the same microvesicle population of interest. As a non-limiting example, the same population of microvesicles detected with KLK2 capture and EpCAM detector can be detected using EpCAM capture and KLK2 detector. Accordingly, the capture/detector pairs indicated in any of **Tables 38, 40-44, 50, 51, 55-67,** and **72-74** can be switched as desired.

[00562] As described, the biosignature can comprise one or more pair of binding agents as desired. In some embodiments, the one or more pair of binding agents comprises binding agents to one or more, e.g., 1, 2 or all, of Mammaglobin - MFG-E8, SIM2 - MFG-E8 and NK-2R - MFG-E8. In another embodiment, the one or more pair of binding agents comprises binding agents to one or more, e.g., 1, 2 or all, of Integrin - MFG-E8, NK-2R - MFG-E8 and Gal3 - MFG-E8. The one or more pair of capture and detector agents may comprise capture agents to one or more, e.g., 1, 2, 3, 4 or all, of AURKB, A33, CD63, Gro-alpha, and Integrin; and detector agents to one or more, e.g., 1, 2, or all, of MUC2, PCSA, and CD8 1. The one or more pair of capture and detector agents may also comprise capture agents to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8 or all, of AURKB, CD63, FLNA, A33, Gro-alpha, Integrin, CD24, SSX2, and SIM2; and detector agents to one or more, e.g., 1, 2, 3, 4 or all, of MUC2, PCSA, CD81, MFG-E8, and EpCam. In some embodiments, the one or more pair of capture and detector agents comprises binding agents to one or more, e.g., 1, 2 or all, of EpCam - MMP7, PCSA - MMP7, and EpCam - BCNP. In some embodiment, the one or more pair of capture and detector agents comprises binding agents to one or more, e.g., 1, 2, 3, 4 or all, of EpCam - MMP7, PCSA - MMP7, EpCam - BCNP, PCSA - ADAM10, and PCSA - KLK2. In still other embodiments, the one or more pair of capture and detector agents comprises binding agents to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCam - MMP7, PCSA - MMP7, EpCam - BCNP, PCSA - ADAM 10, PCSA - KLK2, PCSA - SPDEF, CD81 - MMP7, PCSA - EpCam, MFGE8 - MMP7 and PCSA - IL-8.The one or more pair of capture and detector agents may also comprise binding agents to one or more, e.g., 1, 2, 3, 4 or all, of EpCam - MMP7, PCSA - MMP7, EpCam - BCNP, PCSA - ADAM 10, and CD81 - MMP7.

[00563] The biosignature can comprise one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAM 10, KLK2, SPDEF, CD81, MFGE8, and IL-8. The biosignature can include one or more of these biomarkers as a capture target and/or a detector target. In embodiments, a binding agent to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAM 10, KLK2, SPDEF, CD81, MFGE8, and IL-8 is used to capture a population of vesicles. The captured vesicles can then detected with

PREOMICS EXHIBIT 1002
Page 1087 of 1861

another binding agent to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. Any combination of capture and detector is possible. In one embodiment, the biosignature comprises the following markers: 1) Epcam detector - MMP7 capture; 2) PCSA detector - MMP7 capture; 3) Epcam detector - BCNP capture. In another embodiment, the biosignature comprises the following markers: 1) Epcam detector - MMP7 capture; 2) PCSA detector - MMP7 capture; 3) Epcam detector - BCNP capture; 4) PCSA detector - AdamlO capture; and 5) PCSA detector - KLK2 capture. In still another embodiment, the biosignature comprises the following markers: 1) Epcam detector - MMP7 capture; 2) PCSA detector - MMP7 capture; 3) Epcam detector - BCNP capture; 4) PCSA detector - AdamlO capture; and 5) CD81 detector - MMP7 capture. EpCAM can be used as a detector target when the capture target is one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. MMP7 can be used as a detector target when the capture target is one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. PCSA can be used as a detector target when the capture target is one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. BCNP can be used as a detector target when the capture target is one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. ADAMIO can be used as a detector target when the capture target is one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. KLK2 can be used as a detector target when the capture target is one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. SPDEF can be used as a detector target when the capture target is one or more , e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. CD81 can be used as a detector target when the capture target is one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. MFGE8 can be used as a detector target when the capture target is one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. IL-8 can be used as a detector target when the capture target is one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or all, of EpCAM, MMP7, PCSA, BCNP, ADAMIO, KLK2, SPDEF, CD81, MFGE8, and IL-8. The binding agents can comprise without limitation an antibody, aptamer, or combination thereof. In embodiments, the capture binding agent is tethered to a substrate and the detector binding agent is labeled.

[00564] The biosignature can comprise one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or all, of ADAM- 10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, and SSX4. The biosignature can include one or more of these biomarkers as a capture target and/or a detector target. In embodiments, a binding agent to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or all, of ADAM- 10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, and SSX4 is used to capture a population of vesicles. The captured vesicles can then detected with another binding agent to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or all, of ADAM- 10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, and SSX4. For example, the captured vesicles can be detected with a binding agent to EpCAM. The captured vesicles can be detected with a binding agent to PCSA. The captured vesicles can be detected with a binding agent to ADAM- 10. The captured vesicles can be detected with a binding agent to BCNP. The captured vesicles can be detected with a

PREOMICS EXHIBIT 1002
Page 1088 of 1861

binding agent to CD9. The captured vesicles can be detected with a binding agent to EGFR. The captured vesicles can be detected with a binding agent to IL1B. The captured vesicles can be detected with a binding agent to KLK2. The captured vesicles can be detected with a binding agent to MMP7. The captured vesicles can be detected with a binding agent to p53. The captured vesicles can be detected with a binding agent to PBP. The captured vesicles can be detected with a binding agent to SERPINB3. The captured vesicles can be detected with a binding agent to SPDEF. The captured vesicles can be detected with a binding agent to SSX2. The captured vesicles can be detected with a binding agent to SSX4. In some embodiments, the captured vesicles are detected with a binding agent to one or more of a general vesicle marker, e.g., as described in **Table 3.** The captured vesicles can also be detected with a binding agent to one or more, e.g., 1, 2, 3, 4 or 5, of EpCam, CD81, PCSA, MUC2, and MFG-E8. The captured vesicles can also be detected with a binding agent to one or more tetraspanin, e.g., 1, 2 or 3 of CD9, CD63, CD81, or other tetraspanin as described herein. In some embodiments, the vesicles are captured and detected with one or more pair of binding agents in **Table 72.** The one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or more, pair of binding agents can be selected from the group consisting of EpCAM - EpCAM, EpCAM - KLK2, EpCAM - PBP, EpCAM - SPDEF, EpCAM - SSX2, EpCAM - SSX4, EpCAM - ADAM-10 , EpCAM - SERPINB3, EpCAM - PCSA, EpCAM - p53, EpCAM - MMP7, EpCAM - IL1B, EpCAM - EGFR, EpCAM - CD9, EpCAM - BCNP, KLK2 - EpCAM, KLK2 - KLK2, KLK2 - PBP, KLK2 - SPDEF, KLK2 - SSX2, KLK2 - SSX4, KLK2 - ADAM-10 , KLK2 - SERPINB3, KLK2 - PCSA, KLK2 - p53, KLK2 - MMP7, KLK2 - IL1B, KLK2 - EGFR, KLK2 - CD9, KLK2 - BCNP, PBP - EpCAM, PBP - KLK2, PBP - PBP, PBP - SPDEF, PBP - SSX2, PBP - SSX4, PBP - ADAM-10 , PBP - SERPINB3, PBP - PCSA, PBP - p53, PBP - MMP7, PBP - IL1B, PBP - EGFR, PBP - CD9, PBP - BCNP, SPDEF - EpCAM, SPDEF - KLK2, SPDEF - PBP, SPDEF - SPDEF, SPDEF - SSX2, SPDEF - SSX4, SPDEF - ADAM-10 , SPDEF - SERPINB3, SPDEF - PCSA, SPDEF - p53, SPDEF - MMP7, SPDEF - IL1B, SPDEF - EGFR, SPDEF - CD9, SPDEF - BCNP, SSX2 - EpCAM, SSX2 - KLK2, SSX2 - PBP, SSX2 - SPDEF, SSX2 - SSX2, SSX2 - SSX4, SSX2 - ADAM-10 , SSX2 - SERPINB3, SSX2 - PCSA, SSX2 - p53, SSX2 - MMP7, SSX2 - IL1B, SSX2 - EGFR, SSX2 - CD9, SSX2 - BCNP, SSX4 - EpCAM, SSX4 - KLK2, SSX4 - PBP, SSX4 - SPDEF, SSX4 - SSX2, SSX4 - SSX4, SSX4 - ADAM-10 , SSX4 - SERPINB3, SSX4 - PCSA, SSX4 - p53, SSX4 - MMP7, SSX4 - IL1B, SSX4 - EGFR, SSX4 - CD9, SSX4 - BCNP, ADAM-10 - EpCAM, ADAM-10 - KLK2, ADAM-10 - PBP, ADAM-10 - SPDEF, ADAM-10 - SSX2, ADAM-10 - SSX4, ADAM-10 - ADAM-10 , ADAM-10 - SERPINB3, ADAM-10 - PCSA, ADAM-10 - p53, ADAM-10 - MMP7, ADAM-10 - IL1B, ADAM-10 - EGFR, ADAM-10 - CD9, ADAM-10 - BCNP, SERPINB3 - EpCAM, SERPINB3 - KLK2, SERPINB3 - PBP, SERPINB3 - SPDEF, SERPINB3 - SSX2, SERPINB3 - SSX4, SERPINB3 - ADAM-10 , SERPINB3 - SERPINB3, SERPINB3 - PCSA, SERPINB3 - p53, SERPINB3 - MMP7, SERPINB3 - IL1B, SERPINB3 - EGFR, SERPINB3 - CD9, SERPINB3 - BCNP, PCSA - EpCAM, PCSA - KLK2, PCSA - PBP, PCSA - SPDEF, PCSA - SSX2, PCSA - SSX4, PCSA - ADAM-10 , PCSA - SERPINB3, PCSA - PCSA, PCSA - p53, PCSA - MMP7, PCSA - IL1B, PCSA - EGFR, PCSA - CD9, PCSA - BCNP, p53 - EpCAM, p53 - KLK2, p53 - PBP, p53 - SPDEF, p53 - SSX2, p53 - SSX4, p53 - ADAM-10 , p53 - SERPINB3, p53 - PCSA, p53 - p53, p53 - MMP7, p53 - IL1B, p53 - EGFR, p53 - CD9, p53 - BCNP, MMP7 - EpCAM, MMP7 - KLK2, MMP7 - PBP, MMP7 - SPDEF, MMP7 - SSX2, MMP7 - SSX4, MMP7 - ADAM-10 , MMP7 - SERPINB3, MMP7 - PCSA, MMP7 - p53, MMP7 - MMP7, MMP7 - IL1B, MMP7 - EGFR, MMP7 - CD9, MMP7 - BCNP, IL1B - EpCAM, IL1B -

PREOMICS EXHIBIT 1002
Page 1089 of 1861

KLK2, IL1B - PBP, IL1B - SPDEF, IL1B - SSX2, IL1B - SSX4, IL1B - ADAM-10 , IL1B - SERPINB3, IL1B - PCSA, IL1B - p53, IL1B - MMP7, IL1B - IL1B, IL1B - EGFR, IL1B - CD9, IL1B - BCNP, EGFR - EpCAM, EGFR - KLK2, EGFR - PBP, EGFR - SPDEF, EGFR - SSX2, EGFR - SSX4, EGFR - ADAM-10 , EGFR - SERPINB3, EGFR - PCSA, EGFR - p53, EGFR - MMP7, EGFR - IL1B, EGFR - EGFR, EGFR - CD9, EGFR - BCNP, CD9 - EpCAM, CD9 - KLK2, CD9 - PBP, CD9 - SPDEF, CD9 - SSX2, CD9 - SSX4, CD9 - ADAM-10 , CD9 - SERPINB3, CD9 - PCSA, CD9 - p53, CD9 - MMP7, CD9 - IL1B, CD9 - EGFR, CD9 - CD9, CD9 - BCNP, BCNP - EpCAM, BCNP - KLK2, BCNP - PBP, BCNP - SPDEF, BCNP - SSX2, BCNP - SSX4, BCNP - ADAM-10 , BCNP - SERPINB3, BCNP - PCSA, BCNP - p53, BCNP - MMP7, BCNP - IL1B, BCNP - EGFR, BCNP - CD9, BCNP - BCNP, and a combination thereof, wherein each pair is ordered as the target of the capture - detector agent. The binding agents can be an antibody, aptamer, a combination thereof, or other agent as disclosed herein or known in the art.

[00565] The biosignature can comprise a panel of capture and detector agents. In an embodiment, the panels comprise binding agents to more than one, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or all, of ADAM-10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, and SSX4. For example, the biosignature may comprise a plurality of binding agents selected from the group consisting of SSX4 - EpCAM, SSX4 - KLK2, SSX4 - PBP, SSX4 - SPDEF, SSX4 - SSX2, SSX4 - EGFR, SSX4 - MMP7, SSX4 - BCNP1, SSX4 - SERPINB3, KLK2 - EpCAM, KLK2 - PBP, KLK2 - SPDEF, KLK2 - SSX2, KLK2 - EGFR, KLK2 - MMP7, KLK2 - BCNP1, KLK2 - SERPINB3, PBP - EGFR, PBP - EpCAM, PBP - SPDEF, PBP - SSX2, PBP - SERPINB3, PBP - MMP7, PBP - BCNP1, EpCAM - SPDEF, EpCAM - SSX2, EpCAM - SERPINB3, EpCAM - EGFR, EpCAM - MMP7, EpCAM - BCNP1, SPDEF - SSX2, SPDEF - SERPINB3, SPDEF - EGFR, SPDEF - MMP7, SPDEF - BCNP1, SSX2 - EGFR, SSX2 - MMP7, SSX2 - BCNP1, SSX2 - SERPINB3, SERPINB3 - EGFR, SERPINB3 - MMP7, SERPINB3 - BCNP1, EGFR - MMP7, EGFR - BCNP1, MMP7 - BCNP1, and a combination thereof. The binding agents can be used as capture agents. The captured vesicles can then detected with another binding agent to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or all, of ADAM-10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, and SSX4. For example, the captured vesicles can be detected with a binding agent to EpCAM. In some embodiments, the captured vesicles are detected with a binding agent to one or more of a general vesicle marker, e.g., as described in **Table 3.** The captured vesicles can also be detected with a binding agent to one or more, e.g., 1, 2, 3, 4 or 5, of EpCam, CD81, PCSA, MUC2, and MFG-E8. The captured vesicles can be detected with a binding agent to one or more, e.g., 1, 2, 3, 4, 5 or 6, of CD9, CD63, CD81, PCSA, MUC2, and MFG-E8. The captured vesicles can also be detected with a binding agent to one or more tetraspanin, e.g., 1, 2 or 3 of CD9, CD63, CD81, or other tetraspanin as described herein. In some embodiments, the vesicles are captured and detected with one or more pair of binding agents in **Table 72.** The binding agents can be an antibody, aptamer, a combination thereof, or other agent as disclosed herein or known in the art.

[00566] The biosignature can comprise one or more of EpCAM, KLK2, PBP, SPDEF, SSX2 and SSX4. The biosignature can include one or more of these biomarkers as a capture target and/or a detector target. In embodiments, a binding agent to one or more of EpCAM, KLK2, PBP, SPDEF, SSX2 and SSX4 is used to capture a population of vesicles. The captured vesicles can then detected with another binding agent to one or more of EpCAM, KLK2, PBP, SPDEF, SSX2 and SSX4. For example, captured vesicles can be detected with a binding agent to EpCAM. In an embodiment, the biosignature comprises a microvesicle population detected

PREOMICS EXHIBIT 1002
Page 1090 of 1861

using a binding agent to EpCAM to capture the microvesicles and a binding agent to EpCAM to detect the microvesicles. In an embodiment, the biosignature comprises a microvesicle population detected using a binding agent to KLK2 to capture the microvesicles and a binding agent to EpCAM to detect the microvesicles. In an embodiment, the biosignature comprises a microvesicle population detected using a binding agent to PBP to capture the microvesicles and a binding agent to EpCAM to detect the microvesicles. In an embodiment, the biosignature comprises a microvesicle population detected using a binding agent to SPDEF to capture the microvesicles and a binding agent to EpCAM to detect the microvesicles. In an embodiment, the biosignature comprises a microvesicle population detected using a binding agent to SSX2 to capture the microvesicles and a binding agent to EpCAM to detect the microvesicles. In an embodiment, the biosignature comprises a microvesicle population detected using a binding agent to SSX4 to capture the microvesicles and a binding agent to EpCAM to detect the microvesicles. Any useful combination of these capture/detector pairs can be used as desired. In an embodiment, the combination of capture/detector pairs comprises: 1) EpCAM capture - EpCAM detector; and 2) KLK2, PBP, SPDEF, SSX2 or SSX4 capture - EpCAM detector. In an embodiment, the combination of capture/detector pairs comprises: 1) KLK2 capture - EpCAM detector; and 2) EpCAM, PBP, SPDEF, SSX2 or SSX4 capture - EpCAM detector. In an embodiment, the combination of capture/detector pairs comprises: 1) PBP capture - EpCAM detector; and 2) EpCAM, KLK2, SPDEF, SSX2 or SSX4 capture - EpCAM detector. In an embodiment, the combination of capture/detector pairs comprises: 1) SPDEF capture - EpCAM detector; and 2) EpCAM, KLK2, PBP, SSX2 or SSX4 capture - EpCAM detector. In an embodiment, the combination of capture/detector pairs comprises: 1) SSX2 capture - EpCAM detector; and 2) EpCAM, KLK2, PBP, SPDEF or SSX4 capture - EpCAM detector. In an embodiment, the combination of capture/detector pairs comprises: 1) SSX4 capture - EpCAM detector; and 2) EpCAM, KLK2, PBP, SPDEF or SSX2 capture - EpCAM detector. The binding agents can comprise without limitation an antibody, aptamer, or combination thereof. For example, the capture agents can comprise antibodies and the detector agent can comprise an aptamer. In embodiments, the capture binding agent is tethered to a substrate and the detector binding agent is labeled. If desired, the vesicles can be detected with a binding agent to PCSA.

[00567] In an embodiment, the microvesicles are detecting using capture and detector pairs specific for vesicles from a desired cell of origin. In an embodiment, the vesicles are captured using a cancer marker and detected with a tissue specific marker. Similarly, the vesicles can be captured using a tissue specific marker and detected with a cancer marker. For example, the cancer marker can be EpCAM or B7H3, and the tissue specific marker can be a prostate marker including without limitation PBP, PCSA, PSCA, PSMA, KLK2, PSA, or the like. Without being bound by theory, such embodiments allow for vesicles derived from prostate cancer cells to be detected in circulation.

[00568] Multiple detector agents can be used if desired. For example, the use of multiple general vesicle markers may amplify the detection signal. For example, detection with CD9, CD63 and CD81 together may provide more signal than detection via a single tetraspanin, which may be desirable in some applications.

[00569] In an embodiment, EpCAM (epithelial cellular adhesion molecule) is the target of the anti Epithelial cellular adhesion molecule antibody MAB 9601 in **Table 54.** Further information about EpCAM can be found at www.genecards.org/cgi-bin/carddisp.pl?gene=EPCAM.

[00570] In an embodiment, MMP7 (matrix metallopeptidase 7 (matrilysin, uterine); matrix metalloproteinase 7) is the target of the Anti Matrix metallo Proteinase 7 antibody NB300-1000 in **Table 54.** Further information

PREOMICS EXHIBIT 1002
Page 1091 of 1861

about MMP7 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene=MMP7. Commercially available antibodies to MMP7 that can be used to carry out the methods of the invention include: 1) Anti Matrix metallo Proteinase 7 antibody, R&D Systems, clone 111433, catalog number MAB9071 ; 2) Anti Matrix metallo Proteinase 7 antibody, R&D Systems, clone 111439, catalog number MAB9072; 3) Anti Matrix metallo Proteinase 7 antibody, R&D Systems, clone 6A4, catalog number MAB907; 4) Anti Matrix metallo Proteinase 7 antibody, Millipore, clone 141-7B2, catalog number MAB33 15; 5) Anti Matrix metallo Proteinase 7 antibody, Millipore, clone 176-5F12, MAB3322; 6) Anti Matrix metallo Proteinase 7 polyclonal antibody, Novus, catalog number NB3 00- 1000.

[00571] In an embodiment, PCSA (prostate cell surface antigen) is the target of the Anti prostate cell surface antibody. See **Table 54.** PCSA is also recognized by the 5E10 antibody described in Rokhlin, OW, et al. Cancer Lett., **131:** 129-36 (1998), which publication is incorporated by reference herein in its entirety.

[00572] In an embodiment, BCNP (B-cell novel protein 1; FAM129C; family with sequence similarity 129, member C; niban-like protein 2) is the target of the Anti B-cell novel proteinl antibody ab59781 in **Table 54.** BCNP has several splice forms and isoforms, e.g., BCNP1, BCNP2, BCNP3, BCNP4 and BCNP5. The protein isoforms can also be refered to as Q86XR2-1, Q86XR2-2, Q86XR2-3, Q86XR2-4 and Q86XR2-5. The antibody recognizes at least BCNP1, BCNP2, BCNP3, and may recognize the isoforms 4 and 5. Further information about BCNP is available at www.genecards.org/cgi-bin/carddisp.pl?gene=FAM129C.

[00573] In an embodiment, ADAM10 (ADAM metallopeptidase domain 10; a disintegrin and metalloproteinase domain 10) is the target of the Anti disintegrin and metalloproteinase domain 10 antibody MAB1427 in **Table 54.** Further information about ADAM10 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene=ADAM10.

[00574] In an embodiment, KLK2 (kallikrein-related peptidase 2) is the target of the Anti kallikrein-related peptidase 2 antibody H00003817-M03 in **Table 54.** Further information about KLK2 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene= KLK2.

[00575] In an embodiment, SPDEF (SAM pointed domain containing ets transcription factor) is the target of the Anti SAM pointed domain containing ets transcription factor antibody H00025803-M01 in **Table 54.** Further information about SPDEF can be found at www.genecards.org/cgi-bin/carddisp .pi?gene= SPDEF.

[00576] In an embodiment, CD81 (CD81 molecule; CD81 antigen; tetraspanin-28) is the target of the Anti cluster of differentiation 8 1 antibody 555675 in **Table 54.** Further information about CD81 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene= CD81 .

[00577] In an embodiment, MFGE8 (milk fat globule-EGF factor 8 protein; MFG-E8; sperm associated antigen 10; lactahedrin) is the target of the Anti Milk fat globule-EGF factor 8 protein antibody MAB27671 in **Table 54.** Further information about MFGE8 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene= MFGE8.

[00578] In an embodiment, IL-8 (interleukin 8) is the target of the Anti Interleukin 8 antibody OMA1-03346 in **Table 54.** Further information about IL-8 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene= IL8.

[00579] In an embodiment, SSX4 (synovial sarcoma, X breakpoint 4) is the target of the Anti synovial sarcoma, X breakpoint 4 antibody H00006759-MO2 in **Table 54.** Further information about SSX4 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene=SSX4.

PREOMICS EXHIBIT 1002
Page 1092 of 1861

[00580] In an embodiment, SSX2 (synovial sarcoma, X breakpoint 2) is the target of the Anti synovial sarcoma X break point 2 antibody H00006757-M01 in **Table 54.** Further information about SSX2 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene=SSX2.

[00581] In an embodiment, EGFR (epidermal growth factor receptor) is the target of the Anti epidermal growth factor antibody 555996 in **Table 54.** Further information about EGFR can be found at www.genecards.org/cgi-bin/carddisp.pl?gene=EGFR.

[00582] In an embodiment, SERPINB3 (serpin peptidase inhibitor, clade B (ovalbumin), member 3) is the target of the Anti serpin peptidase inhibitor, clade B member 3 antibody WH00063 17M1 in **Table 54.** Further information about SERPINB3 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene=SERPINB3.

[00583] In an embodiment, IL1B (interleukin 1, beta) is the target of the Anti Interleukin-IB antibody WH0003553M1 in **Table 54.** Further information about IL1B can be found at www.genecards.org/cgi-bin/carddisp.pl?gene= IL1B.

[00584] In an embodiment, TP53 (p53; tumor protein p53) is the target of the Anti tumor protein 53 antibody 654802 in **Table 54.** Further information about TP53 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene=TP53.

[00585] In an embodiment, PBP (prostatic binding protein; PEBP1 ; phosphatidylethanolamine binding protein 1) is the target of the Anti Prostatic binding protein antibody H00005037-M01 in **Table 54.** Further information about PBP can be found at www.genecards.org/cgi-bin/carddisp.pl?gene= PEBP1 .

[00586] In an embodiment, CD9 (CD9 molecule) is the target of the Anti-cluster of differentiation 9 antibody MAB633 in **Table 54.** Further information about CD9 can be found at www.genecards.org/cgi-bin/carddisp.pl?gene= CD9.

[00587] Alternate antibodies, aptamers and other binding agents that recognize the above biomarkers are known in the art. See, e.g., the Genecard references above.

Theranosis

[00588] As disclosed herein, methods are disclosed for characterizing a phenotype for a subject by assessing one or more biomarkers, including vesicle biomarkers and/or circulating biomarkers. The biomarkers can be assessed using methods for multiplexed analysis of vesicle biomarkers disclosed herein. Characterizing a phenotype can include providing a theranosis for a subject, such as determining if a subject is predicted to respond to a treatment or is predicted to be non-responsive to a treatment. A subject that responds to a treatment can be termed a responder whereas a subject that does not respond can be termed a non-responder. A subject suffering from a condition can be considered to be a responder for a treatment based on, but not limited to, an improvement of one or more symptoms of the condition; a decrease in one or more side effects of an existing treatment; an increased improvement, or rate of improvement, in one or more symptoms as compared to a previous or other treatment; or prolonged survival as compared to without treatment or a previous or other treatment. For example, a subject suffering from a condition can be considered to be a responder to a treatment based on the beneficial or desired clinical results including, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. Treatment also includes

PREOMICS EXHIBIT 1002
Page 1093 of 1861

prolonging survival as compared to expected survival if not receiving treatment or if receiving a different treatment.

[00589] The systems and methods disclosed herein can be used to select a candidate treatment for a subject in need thereof. Selection of a therapy can be based on one or more characteristics of a vesicle, such as the biosignature of a vesicle, the amount of vesicles, or both. Vesicle typing or profiling, such as the identification of the biosignature of a vesicle, the amount of vesicles, or both, can be used to identify one or more candidate therapeutic agents for an individual suffering from a condition. For example, vesicle profiling can be used to determine if a subject is a non-responder or responder to a particular therapeutic, such as a cancer therapeutic if the subject is suffering from a cancer.

[00590] Vesicle profiling can be used to provide a diagnosis or prognosis for a subject, and a therapy can be selected based on the diagnosis or prognosis. Alternatively, therapy selection can be directly based on a subject's vesicle profile. Furthermore, a subject's vesicle profile can be used to follow the evolution of a disease, to evaluate the efficacy of a medication, adapt an existing treatment for a subject suffering from a disease or condition, or select a new treatment for a subject suffering from a disease or condition.

[00591] A subject's response to a treatment can be assessed using biomarkers, including vesicles, microRNA, and other circulating biomarkers. In one embodiment, a subject is determined, classified, or identified as a non-responder or responder based on the subject's vesicle profile assessed prior to any treatment. During pretreatment, a subject can be classifed as a non-responder or responder, thereby reducing unnecessary treatment options, and avoidance of possible side effects from ineffective therapeutics. Furthermore, the subject can be identified as a responder to a particular treatment, and thus vesicle profiling can be used to prolong survival of a subject, improve the subject's symptoms or condition, or both, by providing personalized treatment options. Thus, a subject suffering from a condition can have a biosignature generated from vesicles and other circulating biomarkers using one or more systems and methods disclosed herein, and the profile can then be used to determine whether a subject is a likely non-responder or responder to a particular treatment for the condition. Based on use of the biosignature to predict whether the subject is a non-responder or responder to the initially contemplated treatment, a particular treatment contemplated for treating the subject's condition can be selected for the subject, or another potentially more optimal treatment can be selected.

[00592] In one embodiment, a subject suffering from a condition is currently being treated with a therapeutic. A sample can be obtained from the subject before treatment and at one or more timepoints during treatment. A biosignature including vesicles or other biomarkers from the samples can be assessed and used to determine the subject's response to the drug, such as based on a change in the biosignature over time. If the subject is not responding to the treatment, e.g., the biosignature does not indicate that the patient is responding, the subject can be classified as being non-responsive to the treatment, or a non-responder. Similarly, one or more biomarkers associated with a worsening condition may be detected such that the biosignature is indicative of patient's failure to respond favorably to the treatment. In another example, one or more biomarkers associated with the condition remain the same despite treatment, indicating that the condition is not improving. Thus, based on the biosignature, a treatment regimen for the subject can be changed or adapted, including selection of a different therapeutic.

[00593] Alternatively, the subject can be determined to be responding to the treatment, and the subject can be classified as being responsive to the treatment, or a responder. For example, one or more biomarkers associated

PREOMICS EXHIBIT 1002
Page 1094 of 1861

with an improvement in the condition or disorder may be detected. In another example, one or more biomarkers associated with the condition changes, thus indicating an improvement. Thus, the existing treatment can be continued. In another embodiment, even when there is an indiciation of improvement, the existing treatment may be adapted or changed if the biosignature indicates that another line of treatment may be more effective. The existing treatment may be combined with another therapeutic, the dosage of the current therapeutic may be increased, or a different candidate treatment or therapeutic may be selected. Criteria for selecting the different candidate treatment can depend on the setting. In one embodiment, the candidate treatment may have been known to be effective for subjects with success on the existing treatment. In another embodiment, the candidate treatment may have been known to be effective for other subjects with a similar biosignature.

[00594] In some embodiments, the subject is undergoing a second, third or more line of treatment, such as cancer treatment. A biosignature according to the invention can be determined for the subject prior to a second, third or more line of treatment, to determine whether a subject would be a responder or non-resonder to the second, third or more line of treatment. In another embodiment, a biosignature is determined for the subject during the second, third or more line of treatment, to determine if the subject is responding to the second, third or more line of treatment.

[00595] The methods and systems described herein for assessing one or more vesicles can be used to determine if a subject suffering from a condition is responsive to a treatment, and thus can be used to select a treatment that improves one or more symptoms of the condition; decreases one or more side effects of an existing treatment; increases the improvement, or rate of improvement, in one or more symptoms as compared to a previous or other treatment; or prolongs survival as compared to without treatment or a previous or other treatment. Thus, the methods described herein can be used to prolong survival of a subject by providing personalized treatment options, and/or may reduce unnecessary treatment options and unnecessary side effects for a subject.

[00596] The prolonged survival can be an increased progression-free survival (PFS), which denotes the chances of staying free of disease progression for an individual or a group of individuals suffering from a disease, e.g., a cancer, after initiating a course of treatment. It can refer to the percentage of individuals in the group whose disease is likely to remain stable (e.g., not show signs of progression) after a specified duration of time. Progression-free survival rates are an indication of the effectiveness of a particular treatment. In other embodiments, the prolonged survival is disease-free survival (DFS), which denotes the chances of staying free of disease after initiating a particular treatment for an individual or a group of individuals suffering from a cancer. It can refer to the percentage of individuals in the group who are likely to be free of disease after a specified duration of time. Disease-free survival rates are an indication of the effectiveness of a particular treatment. Two treatment strategies can be compared on the basis of the disease-free survival that is achieved in similar groups of patients. Disease-free survival is often used with the term overall survival when cancer survival is described.

[00597] The candidate treatment selected by vesicle profiling as described herein can be compared to a non-vesicle profiling selected treatment by comparing the progression free survival (PFS) using therapy selected by vesicle profiling (period B) with PFS for the most recent therapy on which the subject has just progressed (period A). In one setting, a PFSB/PFSA ratio $\geq$ 1.3 is used to indicate that the vesicle profiling selected therapy provides benefit for subject (see for example, *Robert Temple, Clinical measurement in drug evaluation. Edited*

PREOMICS EXHIBIT 1002
Page 1095 of 1861

by Wu Ningano and G.T. Thicker John Wiley and Sons Ltd. 1995; Von Hoff, D.D. Clin Can Res. 4: 1079, 1999: Dhani et al. Clin Cancer Res. 15: 118-123, 2009).

[00598] Other methods of comparing the treatment selected by vesicle profiling can be compared to a non-vesicle profiling selected treatment by determine response rate (RECIST) and percent of subjects without progression or death at 4 months. The term "about" as used in the context of a numerical value for PFS means a variation of +/- ten percent (10%) relative to the numerical value. The PFS from a treatment selected by vesicle profiling can be extended by at least 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at least 90% as compared to a non- vesicle profiling selected treatment. In some embodiments, the PFS from a treatment selected by vesicle profiling can be extended by at least 100%, 150%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or at least about 1000% as compared to a non-vesicle profiling selected treatment. In yet other embodiments, the PFS ratio (PFS on vesicle profiling selected therapy or new treatment / PFS on prior therapy or treatment) is at least about 1.3. In yet other embodiments, the PFS ratio is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0. In yet other embodiments, the PFS ratio is at least about 3, 4, 5, 6, 7, 8, 9 or 10.

[00599] Similarly, the DFS can be compared in subjects whose treatment is selected with or without determining a biosignature according to the invention. The DFS from a treatment selected by vesicle profiling can be extended by at least 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at least 90% as compared to a non- vesicle profiling selected treatment. In some embodiments, the DFS from a treatment selected by vesicle profiling can be extended by at least 100%, 150%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or at least about 1000% as compared to a non-vesicle profiling selected treatment. In yet other embodiments, the DFS ratio (DFS on vesicle profiling selected therapy or new treatment / DFS on prior therapy or treatment) is at least about 1.3. In yet other embodiments, the DFS ratio is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0. In yet other embodiments, the DFS ratio is at least about 3, 4, 5, 6, 7, 8, 9 or 10.

[00600] In some embodiments, the candidate treatment selected by microvescile profiling does not increase the PFS ratio or the DFS ratio in the subject; nevertheless vesicle profiling provides subject benefit. For example, in some embodiments no known treatment is available for the subject. In such cases, vesicle profiling provides a method to identify a candidate treatment where none is currently identified. The vesicle profiling may extend PFS, DFS or lifespan by at least 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 2 months, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months or 2 years. The vesicle profiling may extend PFS, DFS or lifespan by at least 2 ½ years, 3 years, 4 years, 5 years, or more. In some embodiments, the methods of the invention improve outcome so that subject is in remission.

[00601] The effectiveness of a treatment can be monitored by other measures. A complete response (CR) comprises a complete disappearance of the disease: no disease is evident on examination, scans or other tests. A partial response (PR) refers to some disease remaining in the body, but there has been a decrease in size or number of the lesions by 30% or more. Stable disease (SD) refers to a disease that has remained relatively unchanged in size and number of lesions. Generally, less than a 50% decrease or a slight increase in size would be described as stable disease. Progressive disease (PD) means that the disease has increased in size or number on treatment. In some embodiments, vesicle profiling according to the invention results in a complete response or partial response. In some embodiments, the methods of the invention result in stable disease. In some

PREOMICS EXHIBIT 1002
Page 1096 of 1861

embodiments, the invention is able to achieve stable disease where non-vesicle profiling results in progressive disease.

[00602] The theranosis based on a biosignature of the invention can be for a phenotype including without limitation those listed herein. Characterizing a phenotype includes determining a theranosis for a subject, such as predicting whether a subject is likely to respond to a treatment ("responder") or be non-responsive to a treatment ("non-responder"). As used herein, identifying a subject as a "responder" to a treatment or as a "non-responder" to the treatment comprises identifying the subject as either likely to respond to the treatment or likely to not respond to the treatment, respectively, and does not require determining a definitive prediction of the subject's response. One or more vesicles, or populations of vesicles, obtained from subject are used to determine if a subject is a non-responder or responder to a particular therapeutic, by assessing biomarkers disclosed herein, e.g., those listed in **Table** 7. Detection of a high or low expression level of a biomarker, or a mutation of a biomarker, can be used to select a candidate treatment, such as a pharmaceutical intervention, for a subject with a condtion. **Table** 7 contains illustrative conditions and pharmaceutical interventions for those conditions. The table lists biomarkers that affect the efficacy of the intervention. The biomarkers can be assessed using the methods of the invention, e.g., as circulating biomarkers or in association with a vesicle.

Table 7: **Examples of Biomarkers and Pharmaceutical Intervention for a Condition**

Condition	Pharmaceutial intervention	Biomarker
Peripheral Arterial Disease	Atorvastatin, Simvastatin, Rosuvastatin, Pravastatin, Fluvastatin, Lovastatin	C-reactive protein(CRP), serum Amylyoid A (SAA), interleukin-6, intracellular adhesion molecule (ICAM), vascular adhesion molecule (VCAM), CD40L, fibrinogen, fibrin D-dimer, fibrinopeptide A, von Willibrand factor, tissue plasminogen activator antigen (t-PA), factor VII, prothrombin fragment 1, oxidized low density lipoprotein (oxLDL), lipoprotein A
Non-Small Cell Lung Cancer	Erlotinib, Carboplatin, Paclitaxel, Gefitinib	EGFR, excision repair cross-complementation group 1 (ERCC1), p53, Ras, p27, class III beta tubulin, breast cancer gene 1 (BRCA1), breast cancer gene 1 (BRCA2), ribonucleotide reductase messenger 1 (RRM1)
Colorectal Cancer	Panitumumab, Cetuximab	K-ras
Breast Cancer	Trastuzumab, Anthracyclines, Taxane, Methotrexate, fluorouracil	HER2, toposiomerase II alpha, estrogen receptor, progesterone receptor
Alzheimer's Disease	Donepezil, Galantamine, Memantine, Rivastigmine, Tacrine	beta-amyloid protein, amyloid precursor protein (APP), APP670/671, APP693, APP692, APP715, APP716, APP717, APP723, presenilin 1, presenilin 2, cerebrospinal fluid amyloid beta protein 42 (CSF-Abeta42), cerebrospinal fluid amyloid beta protein 40 (CSF-Abeta40), F2 isoprostane, 4-hydroxynonenal, F4 neuroprostane, acrolein
Arrhythmia	Disopyramide, Flecainide, Lidocaine, Mexiletine,	SERCA, AAP, Connexin 40,

PREOMICS EXHIBIT 1002
Page 1097 of 1861

	Moricizine, Procainamide, Propafenone, Quinidine, Tocainide, Acebutolol, Atenolol, Betaxolol, Bisoprolol, Carvedilol, Esmolol, Metoprolol, Nadolol, Propranolol, Sotalol, Timolol, Amiodarone, Azimilide, Bepridil, Dofetilide, Ibutilide, Tedisamil, Diltiazem, Verapamil, Azimilide, Dronedarone, Amiodarone, PM101, ATI-2042, Tedisamil, Nifekalant, Ambasilide, Ersentilide, Trecetilide, Almokalant, D-sotalol, BRL-32872, HMR1556, L768673, Vernakalant, AZD70009, AVE01 18, S9947, NIP-141/142, XEN-D0101/2, Ranolazine, Pilsicainide, JTV5 19, Rotigaptide, GAP-134	Connexin 43, ATP-sensitive potassium channel, Kvl .5 channel, acetylcholine-activated posassium channel
Rheumatoid arthritis	Methotrexate, infliximab, adalimumab, etanercept, sulfasalazine	677CC/1298AA MTHFR, 677CT/1298AC MTHFR, 677CT MTHFR, G80AA RFC-1, 3435TT MDR1 (ABCB1), 3435TT ABCB1, AMPD1/ATIC/ITPA, IL1-RN3, HLA-DRB103, CRP, HLA-D4, HLA DRB-1, anti-citrulline epitope containing peptides, anti-Al/RA33, Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), SAA (serum amyloid-associated protein), rheumatoid factor, IL-1, TNF, IL-6, IL-8, IL-IRa, Hyaluronic acid, Aggrecan, Glc-Gal-PYD, osteoprotegerin, RNAKL, carilage oligomeric matrix protein (COMP), calprotectin
Arterial Fibrillation	warfarin, aspirin, anticoagulants, heparin, ximelagatran	F1.2, TAT, FPA, beta-throboglobulin, platelet factor 4, soluble P-selectin, IL-6, CRP
HIV Infection	Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Saquinavir, Ritonavir, Indinavir, Nevirane, Nelfinavir, Delavirdine, Stavudine, Efavirenz, Etravirine, Enfuvirtide, Darunavir, Abacavir, Amprenavir, Lonavir/Ritonavirc, Tenofovir, Tipranavir	HIV p24 antigen, TNF-alpha, TNFR-II, CD3, CD14, CD25, CD27, Fas, FasL, beta2 microglobulin, neopterin, HIV RNA, HLA-B *5701
Cardiovascular Disease	lisinopril, candesartan, enalapril	ACE inhibitor, angiotensin

[00603] ***Cancer***

[00604] Vesicle biosignatures can be used in the theranosis of a cancer, such as identifying whether a subject suffering from cancer is a likely responder or non-responder to a particular cancer treatment. The subject methods can be used to theranose cancers including those listed herein, e.g., in the "Phenotype" section above. These include without limitation lung cancer, non-small cell lung cancerm small cell lung cancer (including small cell carcinoma (oat cell cancer), mixed small cell/large cell carcinoma, and combined small cell carcinoma), colon cancer, breast cancer, prostate cancer, liver cancer, pancreatic cancer, brain cancer, kidney cancer, ovarian cancer, stomach cancer, melanoma, bone cancer, gastric cancer, breast cancer, glioma, gliobastoma, hepatocellular carcinoma, papillary renal carcinoma, head and neck squamous cell carcinoma, leukemia, lymphoma, myeloma, or other solid tumors.

[00605] A biosignature of circulating biomarkers, including markers associated with vesicle, in a sample from a subject suffering from a cancer can be used select a candidate treatment for the subject. The biosignature can be

PREOMICS EXHIBIT 1002
Page 1098 of 1861

determined according to the methods of the invention presented herein. In some embodiments, the candidate treatment comprises a standard of care for the cancer. The biosignature can be used to determine if a subject is a non-responder or responder to a particular treatment or standard of care. The treatment can be a cancer treatment such as radiation, surgery, chemotherapy or a combination thereof. The cancer treatment can be a therapeutic such as anti-cancer agents and chemotherapeutic regimens. Cancer treatments for use with the methods of the invention include without limitation those listed in **Table 8**:

Table 8: Cancer Treatments

	Treatment or Agent
Cancer therapies	Radiation, Surgery, Chemotherapy, Biologic therapy, Neo-adjuvant therapy, Adjuvant therapy, Palliative therapy, Watchful waiting
Anti-cancer agents (chemotherapies and biologics)	13-cis-Retinoic Acid, 2-CdA, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 5-FU, 6-Mercaptopurine, 6-MP, 6-TG, 6-Thioguanine, Abraxane, Accutane®, Actinomycin-D, Adriamycin®, Adrucil®, Afinitor®, Agrylin®, Ala-Cort®, Aldesleukin, Alemtuzumab, ALIMTA, Alitretinoin, Alkaban-AQ®, Alkeran®, All-transretinoic Acid, Alpha Interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide, Anagrelide, Anandron®, Anastrozole, Arabinosylcytosine, Ara-C, Aranesp®, Aredia®, Arimidex®, Aromasin®, Arranon®, Arsenic Trioxide, Asparaginase, ATRA, Avastin®, Azacitidine, BCG, BCNU, Bendamustine, Bevacizumab, Bexarotene, BEXXAR®, Bicalutamide, BiCNU, Blenoxane®, Bleomycin, Bortezomib, Busulfan, Busulfex®, C225, Calcium Leucovorin, Campath®, Camptosar®, Camptothecin-11, Capecitabine, Carac™, Carboplatin, Carmustine, Carmustine Wafer, Casodex®, CC-5013, CCI-779, CCNU, CDDP, CeeNU, Cerubidine®, Cetuximab, Chlorambucil, Cisplatin, Citrovorum Factor, Cladribine, Cortisone, Cosmegen®, CPT-11, Cyclophosphamide, Cytadren®, Cytarabine, Cytarabine Liposomal, Cytosar-U®, Cytoxan®, Dacarbazine, Dacogen, Dactinomycin, Darbepoetin Alfa, Dasatinib, Daunomycin Daunorubicin, Daunorubicin Hydrochloride, Daunorubicin Liposomal, DaunoXome®, Decadron, Decitabine, Delta-Cortef®, Deltasone®, Denileukin, Diftitox, DepoCyt™, Dexamethasone, Dexamethasone Acetate Dexamethasone Sodium Phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex Docetaxel, Doxil®, Doxorubicin, Doxorubicin Liposomal, Droxia™, DTIC, DTIC-Dome®, Duralone®, Efudex®, Eligard™, Ellence™, Eloxatin™, Elspar®, Emcyt®, Epirubicin, Epoetin Alfa, Erbitux, Erlotinib, Erwinia L-asparaginase, Estramustine, Ethyol Etopophos®, Etoposide, Etoposide Phosphate, Eulexin®, Everolimus, Evista®, Exemestane, Fareston®, Faslodex®, Femara®, Filgrastim, Floxuridine, Fludara®, Fludarabine, Fluoroplex®, Fluorouracil, Fluorouracil (cream), Fluoxymesterone, Flutamide, Folinic Acid, FUDR®, Fulvestrant, G-CSF, Gefitinib, Gemcitabine, Gemtuzumab ozogamicin, Gemzar, Gleevec™, Gliadel® Wafer, GM-CSF, Goserelin, Granulocyte - Colony Stimulating Factor, Granulocyte Macrophage Colony Stimulating Factor, Halotestin®, Herceptin®, Hexadrol, Hexalen®, Hexamethylmelamine, HMM, Hycamtin®, Hydrea®, Hydrocort Acetate®, Hydrocortisone, Hydrocortisone Sodium Phosphate, Hydrocortisone Sodium Succinate, Hydrocortone Phosphate, Hydroxyurea, Ibritumomab, Ibritumomab, Tiuxetan, Idamycin®, Idarubicin, Ifex®, IFN-alpha, Ifosfamide, IL-11, IL-2, Imatinib mesylate, Imidazole Carboxamide, Interferon alfa, Interferon Alfa-2b (PEG Conjugate), Interleukin-2, Interleukin-11, Intron A® (interferon alfa-2b), Iressa®, Irinotecan, Isotretinoin, Ixabepilone, Ixempra™, Kidrolase (t), Lanacort®, Lapatinib, L-asparaginase, LCR, Lenalidomide, Letrozole, Leucovorin, Leukeran, Leukine™, Leuprolide, Leurocristine, Leustatin™, Liposomal Ara-C Liquid Pred®, Lomustine, L-PAM, L-Sarcolysin, Lupron®, Lupron Depot®, Matulane®, Maxidex, Mechlorethamine, Mechlorethamine Hydrochloride, Medralone®, Medrol®, Megace®, Megestrol, Megestrol Acetate, Melphalan, Mercaptopurine, Mesna, Mesnex™, Methotrexate, Methotrexate Sodium, Methylprednisolone, Meticorten®, Mitomycin, Mitomycin-C, Mitoxantrone, M-Prednisol®, MTC, MTX, Mustargen®, Mustine, Mutamycin®, Myleran®, Mylocel™, Mylotarg®, Navelbine®, Nelarabine, Neosar®, Neulasta™, Neumega®, Neupogen®, Nexavar®, Nilandron®, Nilutamide, Nipent®, Nitrogen Mustard, Novaldex®, Novantrone®, Octreotide, Octreotide acetate, Oncospar®, Oncovin®, Ontak®, Onxal™, Oprevelkin, Orapred®, Orasone®, Oxaliplatin, Paclitaxel, Paclitaxel Protein-bound, Pamidronate, Panitumumab,

PREOMICS EXHIBIT 1002
Page 1099 of 1861

	Panretin®, Paraplatin®, Pediapred®, PEG Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON™, PEG-L-asparaginase, PEMETREXED, Pentostatin, Phenylalanine Mustard, Platinol®, Platinol-AQ®, Prednisolone, Prednisone, Prelone®, Procarbazine, PROCRIT®, Proleukin®, Prolifeprospan 20 with Carmustine Implant, Purinethol®, Raloxifene, Revlimid®, Rheumatrex®, Rituxan®, Rituximab, Roferon-A® (Interferon Alfa-2a), Rubex®, Rubidomycin hydrochloride, Sandostatin®, Sandostatin LAR®, Sargramostim, Solu-Cortef®, Solu-Medrol®, Sorafenib, SPRYCEL™, STI-571, Streptozocin, SU11248, Sunitinib, Sutent®, Tamoxifen, Tarceva®, Targretin®, Taxol®, Taxotere®, Temodar®, Temozolomide, Temsirolimus, Teniposide, TESPA, Thalidomide, Thalomid®, TheraCys®, Thioguanine, Thioguanine Tabloid®, Thiophosphoamide, Thioplex®, Thiotepa, TICE®, Toposar®, Topotecan, Toremifene, Torisel®, Tositumomab, Trastuzumab, Treanda®, Tretinoin, Trexall™, Trisenox®, TSPA, TYKERB®, VCR, Vectibix™, Velban®, Velcade®, VePesid®, Vesanoid®, Viadur™, Vidaza®, Vinblastine, Vinblastine Sulfate, Vincasar Pfs®, Vincristine, Vinorelbine, Vinorelbine tartrate, VLB, VM-26, Vorinostat, VP-16, Vumon®, Xeloda®, Zanosar®, Zevalin™, Zinecard®, Zoladex®, Zoledronic acid, Zolinza, Zometa®
Combination Therapies	CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone); CVP (cyclophosphamide, vincristine, and prednisone); RCVP (Rituximab+CVP); RCHOP (Rituximab+CHOP); RICE (Rituximab+ifosamide, carboplatin, etoposide); RDHAP, (Rituximab+dexamethasone, cytarabine, cisplatin); RESHAP (Rituximab+etoposide, methylprednisolone, cytarabine, cisplatin); combination treatment with vincristine, prednisone, and anthracycline, with or without asparaginase; combination treatment with daunorubicin, vincristine, prednisone, and asparaginase; combination treatment with teniposide and Ara-C (cytarabine); combination treatment with methotrexate and leucovorin; combination treatment with bleomycin, doxorubicin, etoposide, mechlorethamine, prednisone, vinblastine, and vincristine; FOLFOX4 regimen (oxaliplatin, leucovorin, and fluorouracil [5-FU]); FOLFIRI regimen (Irinotecan Hydrochloride, Fluorouracil, and Leucovorin Calcium); Levamisole regimen (5-FU and levamisole); NCCTG regimen (5-FU and low-dose leucovorin); NSABP regimen (5-FU and high-dose leucovorin); XAD (Xelox (Capecitabine + Oxaliplatin) + Bevacizumab + Dasatinib); FOLFOX/Bevacizumab/Hydroxychloroquine; German AIO regimen (folic acid, 5-FU, and irinotecan); Douillard regimen (folic acid, 5-FU, and irinotecan); CAPOX regimen (Capecitabine, oxaliplatin); FOLFOX6 regimen (oxaliplatin, leucovorin, and 5-FU); FOLFIRI regimen (folic acid, 5-FU, and irinotecan); FUFOX regimen (oxaliplatin, leucovorin, and 5-FU); FUOX regimen (oxaliplatin and 5-FU); IFL regimen (irinotecan, 5-FU, and leucovorin); XELOX regimen (capecitabine oxaliplatin); KHAD-L (ketoconazole, hydrocortisone, dutasteride and lapatinib);
Biologies	anti-CD52 antibodies (e.g., Alemtuzumab), anti-CD20 antibodies (e.g., Rituximab), anti-CD40 antibodies (e.g., SGN40)
Classes of Treatments	Anthracyclines and related substances, Anti-androgens, Anti-estrogens, Antigrowth hormones (e.g., Somatostatin analogs), Combination therapy (e.g., vincristine, benu, melphalan, cyclophosphamide, prednisone (VBMCP)), DNA methyltransferase inhibitors, Endocrine therapy - Enzyme inhibitor, Endocrine therapy - other hormone antagonists and related agents, Folic acid analogs (e.g., methotrexate), Folic acid analogs (e.g., pemetrexed), Gonadotropin releasing hormone analogs, Gonadotropin-releasing hormones, Monoclonal antibodies (EGFR-Targeted - e.g., panitumumab, cetuximab), Monoclonal antibodies (Her2-Targeted - e.g., trastuzumab), Monoclonal antibodies (Multi-Targeted - e.g., alemtuzumab), Other alkylating agents, Antineoplastic agents (e.g., asparaginase, ATRA, bexarotene, celecoxib, gemcitabine, hydroxyurea, irinotecan, topotecan, pentostatin), Cytotoxic antibiotics, Platinum compounds, Podophyllotoxin derivatives (e.g., etoposide), Progestogens, Protein kinase inhibitors (EGFR-Targeted), Protein kinase inhibitors (Her2 targeted therapy - e.g., lapatinib), Pyrimidine analogs (e.g., cytarabine), Pyrimidine analogs (e.g., fluoropyrimidines), Salicylic acid and derivatives (e.g., aspirin), Src-family protein tyrosine kinase inhibitors (e.g., dasatinib), Taxanes (e.g., nab-paclitaxel), Vinca Alkaloids and analogs, Vitamin D and analogs, Monoclonal antibodies (Multi-Targeted - e.g., bevacizumab), Protein kinase inhibitors (e.g., imatinib, sorafenib, sunitinib)
Prostate Cancer Treatments	Watchful waiting (i.e., monitor without treatment); Surgery (e.g., Pelvic lymphadenectomy, Radical prostatectomy, Transurethral resection of the prostate (TURP); Orchiectomy); Radiation therapy (e.g., external-beam radiation therapy

PREOMICS EXHIBIT 1002
Page 1100 of 1861

	(EBRT), Proton beam radiation; implantation of radioisotopes (i.e., iodine I 125, palladium, and iridium)); Hormone therapy (e.g., Luteinizing hormone -releasing hormone agonists such as leuprolide, goserelin, buserelin or ozarelix; Antiandrogens such as flutamide, 2-hydroxyflutamide, bicalutamide, megestrol acetate, nilutamide, ketoconazole, aminoglutethimide; calcitriol, gonadotropin-releasing hormone (GnRH), estrogens (DES, chlorotrianisene, ethinyl estradiol, conjugated estrogens USP, and DES-diphosphate), triptorelin, finasteride, cyproterone acetate, ASP3550); Cryosurgery/cryo therapy; Chemotherapy and Biologic therapy (dutasteride, zoledronate, azacitidine, docetaxel, prednisolone, celecoxib, atorvastatin, AMT2003, soy protein, LHRH agonist, PD-103, pomegranate extract, soy extract, taxotere, 1-125, zoledronic acid, dasatinib, vitamin C, vitamin D, vitamin D3, vitamin E, gemcitabine, cisplatin, lenalidomide, prednisone, degarelix, OGX-011, OGX-427, MDV3100, tasquinimod, cabazitaxel, TOOKAD®, lanreotide, PROSTVAC, GM-CSF, lenalidomide, samarium Sm-153 lexidronam, N-Methyl-D-Aspartate (NMDA)-Receptor Antagonist, sorafenib, sorafenib tosylate, mitoxantrone, ABI-008, hydrocortisone, panobinostat, soy-tomato extract, KHAD-L, TOK-001, cixutumumab, temsirolimus, ixabepilone, TAK-700, TAK-448, TRC105, cyclophosphamide, lenalidomide, MLN8237, GDC-0449, Alpharadin®, ARN-509, PX-866, ISIS EIF4E Rx, AEZS-108, 131I-F16SIP Monoclonal Antibody, anti-OX40 antibody, Muscadine Plus, ODM-201, BBI608, ZD4054, erlotinib, rIL-2, epirubicin, estramustine phosphate, HuJ591-GS monoclonal (177Lu-J591), abraxane, IVIG, fermented wheat germ nutriment (FWGE), 153Sm-EDTMP, estramustine, mitoxantrone, vinblastine, carboplatin, paclitaxel, pazopanib, cytarabine, testosterone replacement, Zoledronic Acid, Strontium Chloride Sr 89, paricalcitol, satraplatin, RADOOl (everolimus), valproic acid, tea extract, Hamsa-1, hydroxychloroquine, sipuleucel-T, selenomethionine, selenium, lycopene, sunitinib, vandetanib, IMC-A12 antibody, monoclonal antibody IMC-3G3, ixabepilone, diindolylmethane, metformin, efavirenz, dasatinib, nilutamide, abiraterone, cabozantinib (XL184), isoflavines, cinacalcet hydrochloride, SB939, LY2523355, KX2-391, olaparib, genestein, digoxin, RO4929097, ipilimumab, bafetinib, cediranib maleate, MK2206, phenelzine sulfate, triptorelin pamoate, saracatinib, STA-9090, tesetaxel, pasireotide, afatinib, GTx 758, lonafarnib, satraplatin, radiolabeled antibody 7E11, FP253/fludarabine, Coxsackie A21 (CVA21) virus, ARRY-380, ARRY-382, anti-PSMA designer T cells, pemetrexed disodium, bortezomib, MDX-1 106, white button mushroom extract, SU011248, MLN9708, BMTP-11, ABT-888, CX-4945, 4SC-205, temozolomide, MGAH22, vinorelbine ditartrate, Sodium Selenite, vorinostat, Ad-REIC/Dkk-3, ASG-5ME, IMF-001, PROHIBITIN-TP0 1, DSTP3086S, ridaforolimus, MK-2206, MK-0752, polyunsaturated fatty acids, 1-125, statins, cholecalciferol, omega-3 fatty acids, raloxifene, etoposide, POMELLA™ extract, Lucrin depot); Cancer vaccines (e.g., DNA vaccines, peptide vaccines, dendritic cell vaccines, PEP223, PSA/TRICOM, PROSTVAC-V/TRICOM, PROSTVAC-F/TRICOM, PSA vaccine, TroVax®, GI-6207, PSMA and TARP Peptide Vaccine); Ultrasound; Proton beam radiation
Colorectal Cancer Treatments	Primary Surgical Therapy (e.g., local excision; resection and anastomosis of primary lesion and removal of surrounding lymph nodes); Adjuvant Therapy (e.g., fluorouracil (5-FU), capecitabine, leucovorin, oxaliplatin, erlotinib, irinotecan, aspirin, mitomycin C, suntinib, cetuximab, bevacizumab, pegfilgrastim, panitumumab, ramucirumab, curcumin, celecoxib, FOLFOX4 regimen, FOLFOX6 regimen, FOLFIRI regimen, FUFOX regimen, FUOX regimen, IFL regimen, XELOX regimen, 5-FU and levamisole regimens, German AIO regimen, CAPOX regimen, Douillard regimen, XAD, RADOOl (everolimus), ARQ 197, BMS-908662, JI-101, hydroxychloroquine (HCQ), Yttrium Microspheres, EZN-2208, CS-7017, IMC-1121B, IMC-18F1, docetaxel, lonafarnib, Maytansinoid DM4-Conjugated Humanized Monoclonal Antibody huC242, paclitaxel, ARRY-380, ARRY-382, IMO-2055, MDX1 105-01, CX-4945, Pazopanib, Ixabepilone, OSI-906, NPC-1C Chimeric Monoclonal Antibody, brivanib, Poly-ADP Ribose (PARP) Inhibitor, RO4929097, Anti-cancer vaccine, CEA vaccine, cyclophosphamide, yttrium Y 90 DOTA anti-CEA monoclonal antibody M5A, MEHD7945A, ABT-806, ABT-888, MEDI-565, LY2801653, AZD6244, PRI-724, BKM120, tivozanib, floxuridine, dexamethosone, NKTR-102, perifosine, regorafenib, EP0906, Celebrex, PHY906, KRN330, imatinib mesylate, azacitidine, entinostat, PX-866, ABX-EGF, BAY 43-9006, ESO-1 Lymphocytes and Aldesleukin, LBH589, olaparib, fostamatinib, PD 0332991,

PREOMICS EXHIBIT 1002
Page 1101 of 1861

	STA-9090, cholecalciferol, GI-4000, IL-12, AMG 706, temsirolimus, dulanermin, bortezomib, ursodiol, ridaforolimus, veliparib, NK012, Dalotuzumab, MK-2206, MK-0752, lenalidomide, REOLYSIN®, AUY922, PRI-724, BKM120, avastin, dasatinib); Adjuvant Radiation Therapy (particularly for rectal cancer)

[00606] As shown in **Table 8,** cancer treatments include various surgical and therapeutic treatments. Anti-cancer agents include drugs such as small molecules and biologicals. The methods of the invention can be used to identify a biosignature comprising circulating biomarkers that can then be used for theranostic purposes such as monitoring a treatment efficacy, classifying a subject as a responder or non-responder to a treatment, or selecting a candidate therapeutic agent. The invention can be used to provide a theranosis for any cancer treatments, including without limitation thernosis involving the cancer treatments in **Tables 8-10.** Cancer therapies that can be identified as candidate treatments by the methods of the invention include without limitation the chemotherapeutic agents listed in **Tables 8-10** and any appropriate combinations thereof. In one embodiment, the treatments are specific for a specific type of cancer, such as the treatments listed for prostate cancer, colorectal cancer, breast cancer and lung cancer in **Table 8.** In other embodiments, the treatments are specific for a tumor regardless of its origin but that displays a certain biosignature, such as a biosignature comprising a marker listed in **Tables 9-10.**

[00607] The invention provides methods of monitoring a cancer treatment comprising identifying a series of biosignatures in a subject over a time course, such as before and after a treatment, or over time after the treatment. The biosignatures are compared to a reference to determine the efficacy of the treatment. In an embodiment, the treatment is selected from **Tables 8-10,** such as radiation, surgery, chemotherapy, biologic therapy, neo-adjuvant therapy, adjuvant therapy, or watchful waiting. The reference can be from another individual or group of individuals or from the same subject. For example, a subject with a biosignature indicative of a cancer pre-treatment may have a biosignature indicative of a healthy state after a successful treatment. Conversely, the subject may have a biosignature indicative of cancer after an unsuccessful treatment. The biosignatures can be compared over time to determine whether the subject's biosignatures indicate an improvement, worsening of the condition, or no change. Additional treatments may be called for if the cancer is worsening or there is no change over time. For example, hormone therapy may be used in addition to surgery or radiation therapy to treat more aggressive prostate cancers. One or more of the following miRs can be used in a biosignature for monitoring an efficacy of prostate cancer treatment: hsa-miR-1974, hsa-miR-27b, hsa-miR-103, hsa-miR-146a, hsa-miR-22, hsa-miR-382, hsa-miR-23a, hsa-miR-376c, hsa-miR-335, hsa-miR-142-5p, hsa-miR-221, hsa-miR-142-3p, hsa-miR-15 1-3p, hsa-miR-21, hsa-miR-16. One or more miRs listed in the following publication can be used in a biosignature for monitoring treatment of a cancer of the GI tract: Albulescu et al., Tissular and soluble miRNAs for diagnostic and therapy improvement in digestive tract cancers, Exp Rev Mol Diag, **11**:1, 101-120.

[00608] In some embodiments, the invention provides a method of identifying a biosignature in a sample from a subject in order to select a candidate therapeutic. For example, the biosignature may indicate that a drug-associated target is mutated or differentially expressed, thereby indicating that the subject is likely to respond or not respond to certain treatments. The candidate treatments can be chosen from the anti-cancer agents or classes of therapeutic agents identified in **Tables 8-10.** In some embodiments, the candidate treatments identified according to the subject methods are chosen from at least the groups of treatments consisting of 5-fluorouracil, abarelix, alemtuzumab, aminoglutethimide, anastrozole, asparaginase, aspirin, ATRA, azacitidine, bevacizumab,

PREOMICS EXHIBIT 1002
Page 1102 of 1861

bexarotene, bicalutamide, calcitriol, capecitabine, carboplatin, celecoxib, cetuximab, chemotherapy, cholecalciferol, cisplatin, cytarabine, dasatinib, daunorubicin, decitabine, doxorubicin, epirubicin, erlotinib, etoposide, exemestane, flutamide, fulvestrant, gefitinib, gemcitabine, gonadorelin, goserelin, hydroxyurea, imatinib, irinotecan, lapatinib, letrozole, leuprolide, liposomal-doxorubicin, medroxyprogesterone, megestrol, megestrol acetate, methotrexate, mitomycin, nab-paclitaxel, octreotide, oxaliplatin, paclitaxel, panitumumab, pegaspargase, pemetrexed, pentostatin, sorafenib, sunitinib, tamoxifen, taxanes, temozolomide, toremifene, trastuzumab, VBMCP, and vincristine.

[00609] Similar to selecting a candidate treatment, the invention also provides a method of determining whether to treat a cancer at all. For example, prostate cancer can be a non-aggressive disease that is unlikely to substantially harm the subject. Radiation therapy with androgen ablation (hormone reduction) is the standard method of treating locally advanced prostate cancer. Morbidities of hormone therapy include impotence, hot flashes, and loss of libido. In addition, a treatment such as prostatectomy can have morbidities such as impotence or incontinence. Therefore, the invention provides biosignatures that indicate aggressiveness or a progression (e.g., stage or grade) of the cancer. A non-aggressive cancer or localized cancer might not require immediate treatment but rather be watched, e.g., "watchful waiting" of a prostate cancer. Whereas an aggressive or advanced stage lesion would require a concomitantly more aggressive treatment regimen.

[00610] Examples of biomarkers that can be detected, and treatment agents that can be selected or possibly avoided are listed in **Table 9.** For example, a biosignature is identified for a subject with a prostate cancer, wherein the biosignature comprises levels of androgen receptor (AR). Overexpression or overproduction of AR, such as high levels of mRNA levels or protein levels in a vesicle, provides an identification of candidate treatments for the subject. Such treatments include agents for treating the subject such as Bicalutamide, Flutamide, Leuprolide, or Goserelin. The subject is accordingly identified as a responder to Bicalutamide, Flutamide, Leuprolide, or Goserelin. In another illustrative example, BCRP mRNA, protein, or both is detected at high levels in a vesicle from a subject suffering from NSCLC. The subject may then be classified as a non-responder to the agents Cisplatin and Carboplatin, or the agents are considered to be less effective than other agents for treating NSCLC in the subject and not selected for use in treating the subject. Any of the following biomarkers can be assessed in a vesicle obtained from a subject, and the biomarker can be in the form including but not limited to one or more of a nucleic acid, polypeptide, peptide or peptide mimetic. In yet another illustrative example, a mutation in one or more of KRAS, BRAF, PIK3CA, and/or c-kit can be used to select a candidate treatment. For example, a mutation in KRAS or BRAF in a patient may indicate that cetuximab and/or panitumumab are likely to be less effective in treating the patient.

Table 9: Examples of Biomarkers, Lineage and Agents

Biomarker	Lineage	Possibly Less Effective Agents	Possible Agents to Consider
AR (high expression)	Prostate		Bicalutamide, Flutamide, Leuprolide, Goserelin
AR (high expression)	default		Bicaluamide, Flutamide, Leuprolide, Goserelin
BCRP (high expression)	Non-small cell lung cancer (NSCLC)	Cisplatin, Carboplatin	
BCRP (low expression)	Non-small cell lung cancer (NSCLC)		Cisplatin, Carboplatin
BCRP (high expression)	default	Cisplatin, Carboplatin	

PREOMICS EXHIBIT 1002
Page 1103 of 1861

BCRP (low expression)	default		Cisplatin, Carboplatin
BRAF V600E (mutation positive)	Colorectal	Cetuximab, Panitumumab	
BRAF V600E (mutation negative)	Colorectal		Cetuximab, Panitumumab
BRAF V600E (mutation positive)	All other	Cetuximab, Panitumumab	
BRAF V600E (mutation negative)	All other		Cetuximab, Panitumumab
BRAF V600E (mutation positive)	default	Cetuximab, Panitumumab	
BRAF V600E (mutation negative)	default		Cetuximab, Panitumumab
CD52 (high expression)	Leukemia		Alemtuzumab
CD52 (low expression)	Leukemia	Alemtuzumab	
CD52 (high expression)	default (Hematologic malignancies only)		Alemtuzumab
CD52 (low expression)	default (Hematologic malignancies only)	Alemtuzumab	
c-kit	Uveal Melanoma		
c-kit (high expression)	Gastrointestinal Stromal Tumors [GIST]; cKIT will not be performed on Uveal Melanoma as imatinib is not useful in the setting of WT cKIT positive uveal melanoma (see Hofmann et al. 2009)		Imatinib
c-kit (high expression)	Extrahepatic Bile Duct Tumors; cKIT will not be performed on Uveal Melanoma as imatinib is not useful in the setting of WT cKIT positive uveal melanoma (see Hofmann et al. 2009)		Imatinib
c-kit (high expression)	Acute myeloid leukemia (AML)		Imatinib
c-kit (high expression)	default; cKIT will not be performed on Uveal Melanoma as imatinib is not useful in the setting of WT cKIT positive uveal melanoma (see Hofmann et al. 2009)		Imatinib
EGFR (high copy number)	Head and neck squamous cell carcinoma (HNSCC)		Erlotinib, Gefitinib
EGFR	Head and neck squamous cell carcinoma (HNSCC)	Erlotinib, Gefitinib	
EGFR (high copy number)	Non-small cell lung cancer (NSCLC)		Erlotinib, Gefitinib
EGFR (low copy number)	Non-small cell lung cancer (NSCLC)	Erlotinib, Gefitinib	
EGFR (high copy number)	default		Cetuxumab, Panitumumab, Erlotinib, Gefitinib
EGFR (low copy	default	Cetuxumab, Panitumumab,	

PREOMICS EXHIBIT 1002
Page 1104 of 1861

number)		Erlotinib, Gefitinib	
ER (high expression)	Breast	Ixabepilone	Tamoxifen-based treatment, aromatase inhibitors (anastrazole, letrozole)
ER (low expression)	Breast		Ixabepilone
ER (high expression)	Ovarian		Tamoxifen-based treatment, aromatase inhibitors (anastrazole, letrozole)
ER (high expression)	default		Tamoxifen-based treatment, aromatase inhibitors (anastrazole, letrozole)
ERCC1 (high expression)	Non-small cell lung cancer (NSCLC)	Carboplatin, Cisplatin	
ERCC1 (low expression)	Non-small cell lung cancer (NSCLC)		Carboplatin, Cisplatin
ERCC1 (high expression)	Small Cell Lung Cancer (SCLC)	Carboplatin, Cisplatin	
ERCC1 (low expression)	Small Cell Lung Cancer (SCLC)		Carboplatin, Cisplatin
ERCC1 (high expression)	Gastric	Oxaliplatin	
ERCC1 (low expression)	Gastric		Oxaliplatin
ERCC1 (high expression)	default	Carboplatin, Cisplatin, Oxaliplatin	
ERCC1 (low expression)	default		Carboplatin, Cisplatin, Oxaliplatin
HER-2 (high expression)	Breast		Lapatinib, Trastuzumab
HER-2 (high expression)	default		Lapatinib, Trastuzumab
KRAS (mutation positive)	Colorectal cancer	Cetuximab, Panitumumab	
KRAS (mutation negative)	Colorectal cancer		Cetuximab, Panitumumab
KRAS (mutation positive)	Non-small cell lung cancer (NSCLC)	Erlotinib, Gefitinib	
KRAS (mutation negative)	Non-small cell lung cancer (NSCLC)		Erlotinib, Gefitinib
KRAS (mutation positive)	Bronchioloalveolar carcinoma (BAC) or adenocarcinoma (BAC subtype)	Erlotinib	
KRAS (mutation negative)	Bronchioloalveolar carcinoma (BAC) or adenocarcinoma (BAC subtype)		Erlotinib
KRAS (mutation positive)	Multiple myeloma	VBMCP/Cyclophosphamide	
KRAS (mutation negative)	Multiple myeloma		VBMCP/Cyclophosphamide
KRAS (mutation positive)	default	Cetuximab, Panitumumab	
KRAS (mutation negative)	default		Cetuximab, panitumumab
KRAS (mutation positive)	default	Cetuximab, Erlotinib, Panitumumab, Gefitinib	
KRAS (mutation negative)	default		Cetuximab, Erlotinib, Panitumumab, Gefitinib

PREOMICS EXHIBIT 1002
Page 1105 of 1861

MGMT (high expression)	Pituitary tumors, oligodendroglioma	Temozolomide	
MGMT (low expression)	Pituitary tumors, oligodendroglioma		Temozolomide
MGMT (high expression)	Neuroendocrine tumors	Temozolomide	
MGMT (low expression)	Neuroendocrine tumors		Temozolomide
MGMT (high expression)	default	Temozolomide	
MGMT (low expression)	default		Temozolomide
MRP1 (high expression)	Breast	Cyclophosphamide	
MRP1 (low expression)	Breast		Cyclophosphamide
MRP1 (high expression)	Small Cell Lung Cancer (SCLC)	Etoposide	
MRP1 (low expression)	Small Cell Lung Cancer (SCLC)		Etoposide
MRP1 (high expression)	Nodal Diffuse Large B-Cell Lymphoma	Cyclophosphamide/Vincristine	
MRP1 (low expression)	Nodal Diffuse Large B-Cell Lymphoma		Cyclophosphamide/Vincristine
MRP1 (high expression)	default	Cyclophosphamide, Etoposide, Vincristine	
MRP1 (low expression)	default		Cyclophosphamide, Etoposide, Vincristine
PDGFRA (high expression)	Malignant Solitary Fibrous Tumor of the Pleura (MSFT)		Imatinib
PDGFRA (high expression)	Gastrointestinal stromal tumor (GIST)		Imatinib
PDGFRA (high expression)	Default		Imatinib
p-glycoprotein (high expression)	Acute myeloid leukemia (AML)	Etoposide	
p-glycoprotein (low expression)	Acute myeloid leukemia (AML)		Etoposide
p-glycoprotein (high expression)	Diffuse Large B-cell Lymphoma (DLBCL)	Doxorubicin	
p-glycoprotein (low expression)	Diffuse Large B-cell Lymphoma (DLBCL)		Doxorubicin
p-glycoprotein (high expression)	Lung	Etoposide	
p-glycoprotein (low expression)	Lung		Etoposide
p-glycoprotein (high expression)	Breast	Doxorubicin	
p-glycoprotein (low expression)	Breast		Doxorubicin
p-glycoprotein (high expression)	Ovarian	Paclitaxel	
p-glycoprotein (low expression)	Ovarian		Paclitaxel
p-glycoprotein (high expression)	Head and neck squamous cell carcinoma (HNSCC)	Vincristine	
p-glycoprotein (low expression)	Head and neck squamous cell carcinoma (HNSCC)		Vincristine

PREOMICS EXHIBIT 1002
Page 1106 of 1861

p-glycoprotein (high expression)	default	Vincristine, Etoposide, Doxorubicin, Paclitaxel	
p-glycoprotein (low expression)	default		Vincristine, Etoposide, Doxorubicin, Paclitaxel
PR (high expression)	Breast	Chemoendocrine therapy	Tamoxifen, Anastrazole, Letrozole
PR (low expression)	default	Chemoendocrine therapy	Tamoxifen, Anastrazole, Letrozole
PTEN (high expression)	Breast		Trastuzumab
PTEN (low expression)	Breast	Trastuzumab	
PTEN (high expression)	Non-small cell Lung Cancer (NSCLC)		Gefitinib
PTEN (low expression)	Non-small cell Lung Cancer (NSCLC)	Gefitinib	
PTEN (high expression)	Colorectal		Cetuximab, Panitumumab
PTEN (low expression)	Colorectal	Cetuximab, Panitumumab	
PTEN (high expression)	Glioblastoma		Erlotinib, Gefitinib
PTEN (low expression)	Glioblastoma	Erlotinib, Gefitinib	
PTEN (high expression)	default		Cetuximab, Panitumumab, Erlotinib, Gefitinib and Trastuzumab
PTEN (low expression)	default	Cetuximab, Panitumumab, Erlotinib, Gefitinib and Trastuzumab	
RRM1 (high experssion)	Non-small cell lung cancer (NSCLC)	Gemcitabine	
RRM1 (low expression)	Non-small cell lung cancer (NSCLC)		Gemcitabine
RRM1 (high experssion)	Pancreas	Gemcitabine	
RRM1 (low expression)	Pancreas		Gemcitabine
RRM1 (high experssion)	default	Gemcitabine	
RRM1 (low expression)	default		Gemcitabine
SPARC (high expression)	Breast		nab-paclitaxel
SPARC (high expression)	default		nab-paclitaxel
TS (high expression)	Colorectal	fluoropyrimidines	
TS (low expression)	Colorectal		fluoropyrimidines
TS (high expression)	Pancreas	fluoropyrimidines	
TS (low expression)	Pancreas		fluoropyrimidines
TS (high expression)	Head and Neck Cancer	fluoropyrimidines	
TS (low expression)	Head and Neck Cancer		fluoropyrimidines
TS (high expression)	Gastric	fluoropyrimidines	
TS (low expression)	Gastric		fluoropyrimidines
TS (high expression)	Non-small cell lung cancer (NSCLC)	fluoropyrimidines	
TS (low expression)	Non-small cell lung cancer (NSCLC)		fluoropyrimidines
TS (high expression)	Liver	fluoropyrimidines	

PREOMICS EXHIBIT 1002
Page 1107 of 1861

TS (low expression)	Liver		fluoropyrimidines
TS (high expression)	default	fluoropyrimidines	
TS (low expression)	default		fluoropyrimidines
TOPOl (high expression)	Colorectal		Irinotecan
TOPOl (low expression)	Colorectal	Irinotecan	
TOPOl (high expression)	Ovarian		Irinotecan
TOPOl (low expression)	Ovarian	Irinotecan	
TOPOl (high expression)	default		Irinotecan
TOPOl (low expression)	default	Irinotecan	
TopoIIa (high epxression)	Breast		Doxorubicin, liposomal-Doxorubicin, Epirubicin
TopoIIa (low expression)	Breast	Doxorubicin, liposomal-Doxorubicin, Epirubicin	
TopoIIa (high epxression)	default		Doxorubicin, liposomal-Doxorubicin, Epirubicin
TopoIIa (low expression)	default	Doxorubicin, liposomal-Doxorubicin, Epirubicin	

[00611] Other examples of biomarkers that can be detected and the treatment agents that can be selected or possibly avoided based on the biomarker signatures are listed in **Table 10.** For example, for a subject suffering from cancer, detecting overexpression of ADA in vesicles from a subject is used to classify the subject as a responder to pentostatin, or pentostatin identified as an agent to use for treating the subject. In another example, for a subject suffering from cancer, detecting overexpression of BCRP in vesicles from the subject is used to classify the subject as a non-responder to cisplatin, carboplatin, irinotecan, and topotecan, meaning that cisplatin, carboplatin, irinotecan, and topotecan are identified as agents that are suboptimal for treating the subject.

Table 10: Examples of Biomarkers, Agents and Resistance

Gene Name	Expression Status	Candidate Agent(s)	Possible Resistance
ADA	Overexpressed	pentostatin	
ADA	Underexpressed		cytarabine
AR	Overexpressed	abarelix, bicalutamide, flutamide, gonadorelin, goserelin, leuprolide	
ASNS	Underexpressed	asparaginase, pegaspargase	
BCRP (ABCG2)	Overexpressed		cisplatin, carboplatin, irinotecan, topotecan
BRAF	Mutated		panitumumab, cetuximmab
BRCA1	Underexpressed	mitomycin	
BRCA2	Underexpressed	mitomycin	
CD52	Overexpressed	alemtuzumab	
CDA	Overexpressed		cytarabine
c-erbB2	High levels of phosphorylation in epithelial cells	Trastuzumab, c-erbB2 kinase inhibitor, lapatinib	
CES2	Overexpressed	irinotecan	
c-kit	Overexpressed	sorafenib, sunitinib, imatinib	

PREOMICS EXHIBIT 1002
Page 1108 of 1861

COX-2	Overexpressed	celecoxib	
DCK	Overexpressed	gemcitabine	cytarabine
DHFR	Underexpressed	methotrexate, pemetrexed	
DHFR	Overexpressed		methotrexate
DNMT1	Overexpressed	azacitidine, decitabine	
DNMT3A	Overexpressed	azacitidine, decitabine	
DNMT3B	Overexpressed	azacitidine, decitabine	
EGFR	Overexpressed	erlotinib, gefitinib, cetuximab, panitumumab	
EML4-ALK	Overexpressed (present)	petrexmed, crizotinib	
EPHA2	Overexpressed	dasatinib	
ER	Overexpressed	anastrazole, exemestane, fulvestrant, letrozole, megestrol, tamoxifen, medroxyprogesterone, toremifene, aminoglutethimide	
ERCC1	Overexpressed		carboplatin, cisplatin, oxaliplatin
GART	Underexpressed	pemetrexed	
GRN (PCDGF, PGRN)	Overexpressed		anti-oestrogen therapy, tamoxifen, faslodex, letrozole, herceptin in Her-2 overexpressing cells, doxorubicin
HER-2 (ERBB2)	Overexpressed	trastuzumab, lapatinib	
HIF-la	Overexpressed	sorafenib, sunitinib, bevacizumab	
IκB-α	Overexpressed	bortezomib	
IGFBP3	Underexpressed	letrozole	
IGFBP4	Overexpressed	letrozole	
IGFBP5	Underexpressed	letrozole	
Ki67	Underexpressed	tamoxifen + chemotherapy	
KRAS	Mutated		panitumumab, cetuximab
MET	Overexpressed		gefitinib, erlotinib
MGMT	Underexpressed	temozolomide	
MGMT	Overexpressed		temozolomide
MRP1 (ABCC1)	Overexpressed		etoposide, paclitaxel, docetaxel, vinblastine, vinorelbine, topotecan, teniposide
P-gp (ABCBl)	Overexpressed		doxorubicin, etoposide, epirubicin, paclitaxel, docetaxel, vinblastine, vinorelbine, topotecan, teniposide, liposomal doxorubicin
PDGFR-a	Overexpressed	sorafenib, sunitinib, imatinib	
PDGFR-β	Overexpressed	sorafenib, sunitinib, imatinib	
PIK3CA/PI3K	Mutation		cetuximab, panitumumab, trastuzumab
PR	Overexpressed	exemestane, fulvestrant, gonadorelin, goserelin, medroxyprogesterone, megestrol, tamoxifen,	

PREOMICS EXHIBIT 1002
Page 1109 of 1861

		toremifene	
PTEN	Underexpressed		cetuximab, panitumumab, trastuzumab
RARA	Overexpressed	ATRA	
RRM1	Underexpressed	gemcitabine, hydroxyurea	
RRM2	Underexpressed	gemcitabine, hydroxyurea	
RRM2B	Underexpressed	gemcitabine, hydroxyurea	
RXR-a	Overexpressed	bexarotene	
RXR-β	Overexpressed	bexarotene	
SPARC	Overexpressed	nab-paclitaxel	
SRC	Overexpressed	dasatinib	
SSTR2	Overexpressed	octreotide	
SSTR5	Overexpressed	octreotide	
TLE3			
TOPO I	Overexpressed	irinotecan, topotecan	
TOPO IIa	Overexpressed	doxorubicin, epirubicin, liposomal- doxorubicin	
TOPO IIβ	Overexpressed	doxorubicin, epirubicin, liposomal- doxorubicin	
TS	Underexpressed	capecitabine, 5-fluorouracil, pemetrexed	
TS	Overexpressed		capecitabine, 5-fluorouracil
TUBB3	Overexpressed		paclitaxel, docetaxel
VDR	Overexpressed	calcitriol, cholecalciferol	
VEGFR1 (Fltl)	Overexpressed	sorafenib, sunitinib, bevacizumab	
VEGFR2	Overexpressed	sorafenib, sunitinib, bevacizumab	
VHL	Underexpressed	sorafenib, sunitinib	

[00612] Further drug associations and rules that are used in embodiments of the invention are found in U.S. Patent Application 12/658,770, filed February 12, 2010; and International PCT Patent Applications PCT/US20 10/000407, filed February 11, 2010; PCT/US20 10/543 66, filed October 27, 2010; PCT/US201 1/067527, filed December 28, 201 1; and PCT/US2012/041393, filed June 7, 2012, all of which applications are incorporated by reference herein in their entirety. See, e.g., "Table 4: Rules Summary for Treatment Selection" of PCT/US2010/54366; "Table 5: Rules Summary for Treatment Selection" of PCT/US20 11/067527; and Tables 7-12 of PCT/US2012/041393.

[00613] Any drug-associated target can be part of a biosignature for providing a theranosis. A "druggable target" comprising a target that can be modulated with a therapeutic agent such as a small molecule or biologic, is a candidate for inclusion in the biosignature of the invention. Drug-associated targets also include biomarkers that can confer resistance to a treatment, such as shown in **Tables 9** and **10.** The biosignature can be based on either the gene, e.g., DNA sequence, and/or gene product, e.g., mRNA or protein, or the drug-associated target. Such nucleic acid and/or polypeptide can be profiled as applicable as to presence or absence, level or amount, activity, mutation, sequence, haplotype, rearrangement, copy number, or other measurable characteristic. The gene or gene product can be associated with a vesicle population, e.g., as a vesicle surface marker or as vesicle payload. In an embodiment, the invention provides a method of theranosing a cancer, comprising identifying a biosignature that comprises a presence or level of one or more drug-associated target, and selecting a candidate therapeutic based on the biosignature. The drug-associated target can be a circulating biomarker, a vesicle, or a

PREOMICS EXHIBIT 1002
Page 1110 of 1861

vesicle associated biomarker. Because drug-associated targets can be independent of the tissue or cell-of-origin, biosignatures comprising drug-associated targets can be used to provide a theranosis for any proliferative disease, such as cancers from various anatomical origins, including cancers of unknown origin such as CUPS.

[00614] The drug-associated targets assessed using the methods of the invention comprise without limitation ABCC1, ABCG2, ACE2, ADA, ADH1C, ADH4, AGT, AR, AREG, ASNS, BCL2, BCRP, BDCA1, beta III tubulin, BIRC5, B-RAF, BRCA1, BRCA2, CA2, caveolin, CD20, CD25, CD33, CD52, CDA, CDKN2A, CDKN1A, CDKN1B, CDK2, CDW52, CES2, CK 14, CK 17, CK 5/6, c-KIT, c-Met, c-Myc, COX-2, Cyclin Dl, DCK, DHFR, DNMT1, DNMT3A, DNMT3B, E-Cadherin, ECGF1, EGFR, EML4-ALK fusion, EPHA2, Epiregulin, ER, ERBR2, ERCC1, ERCC3, EREG, ESR1, FLT1, folate receptor, FOLR1, FOLR2, FSHB, FSHPRH1, FSHR, FYN, GART, GNA1 1, GNAQ, GNRH1, GNRHR1, GSTP1, HCK, HDAC1, hENT-1, Her2/Neu, HGF, HIF1A, HIG1, HSP90, HSP90AA1, HSPCA, IGF-1R, IGFRBP, IGFRBP3, IGFRBP4, IGFRBP5, IL13RA1, IL2RA, KDR, Ki67, KIT, K-RAS, LCK, LTB, Lymphotoxin Beta Receptor, LYN, MET, MGMT, MLH1, MMR, MRP1, MS4A1, MSH2, MSH5, Myc, NFKB1, NFKB2, NFKBIA, NRAS, ODC1, OGFR, pl6, p21, p27, p53, p95, PARP-1, PDGFC, PDGFR, PDGFRA, PDGFRB, PGP, PGR, PI3K, POLA, POLA1, PPARG, PPARGC1, PR, PTEN, PTGS2, PTPN12, RAF 1, RARA, ROS1, RRM1, RRM2, RRM2B, RXRB, RXRG, SIK2, SPARC, SRC, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, Survivin, TK1, TLE3, TNF, TOPI, TOP2A, TOP2B, TS, TUBB3, TXN, TXNRD1, TYMS, VDR, VEGF, VEGFA, VEGFC, VHL, YES1, ZAP70, or any combination thereof. A biosignature including one or combination of these markers can be used to characterize a phenotype according to the invention, such as providing a theranosis. These markers are known to play a role in the efficacy of various chemotherapeutic agents against proliferative diseases. Accordingly, the markers can be assessed to select a candidate treatment for the cancer independent of the origin or type of cancer. In an embodiment, the invention provides a method of selecting a candidate therapeutic for a cancer, comprising identifying a biosignature comprising a level or presence of one or more drug associated target, and selecting the candidate therapeutic based on its predicted efficacy for a patient with the biosignature. The one or more drug-associated target can be one of the targets listed above, or in **Tables 9-10.** In some embodiments, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 25, 30, 35, 40, 45, or at least 50 of the one or more drug-associated targets are assessed. The one or more drug-associated target can be associated with a vesicle, e.g., as a vesicle surface marker or as vesicle payload as either nucleic acid (e.g., DNA, mRNA) or protein. In some embodiments, the presence or level of a microRNA known to interact with the one or more drug-associated target is assessed, wherein a high level of microRNA known to suppress the one or more drug-associated target can indicate a lower expression of the one or more drug-associated target and thus a lower likelihood of response to a treatment against the drug-associated target. The one or more drug-associated target can be circulating biomarkers. The one or more drug-associated target can be assessed in a tissue sample. The predicted efficacy can be determined by comparing the presence or level of the one or more drug-associated target to a reference value, wherein a higher level that the reference indicates that the subject is a likely responder. The predicted efficacy can be determined using a classifier algorithm, wherein the classifier was trained by comparing the biosignature of the one or more drug-associated target in subjects that are known to be responders or non-responders to the candidate treatment. Molecular associations of the one or more drug-associated target with appropriate candidate targets are displayed in **Tables 9-10** herein and U.S. Patent Application 12/658,770, filed February 12, 2010; International PCT Patent Application PCT/US20 10/000407, filed February 11, 2010;

PREOMICS EXHIBIT 1002
Page 1111 of 1861

International PCT Patent Application PCT/US20 10/543 66, filed October 27, 2010; International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1; International Patent Application Serial No. PCT/US20 11/067527, entitled "MOLECULAR PROFILING OF CANCER" and filed December 28, 201 1; and U.S. Provisional Patent Application 61/427,788, filed December 28, 2010; all of which applications are incorporated by reference herein in their entirety.

[00615] Table 11 of International Patent Application Serial No. PCT/US201 1/03 1479, provides a listing of gene and corresponding protein symbols and names of many of the theranostic targets that are analyzed according to the methods of the invention. As understood by those of skill in the art, genes and proteins have developed a number of alternative names in the scientific literature. Thus, the listing in Table 11 of PCT/US201 1/03 1479 and Table 2 of PCT/US201 1/067527 comprise illustrative but not exhaustive compilations. A further listing of gene aliases and descriptions can be found using a variety of online databases, including GeneCards® (www.genecards.org), HUGO Gene Nomenclature (www.genenames.org), Entrez Gene (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene), UniProtKB/Swiss-Prot (www.uniprot.org), UniProtKB/TrEMBL (www.uniprot.org), OMIM (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM), GeneLoc (genecards.weizmann.ac.il/geneloc/), and Ensembl (www.ensembl.org). Generally, gene symbols and names below correspond to those approved by HUGO, and protein names are those recommended by UniProtKB/Swiss-Prot. Common alternatives are provided as well. Where a protein name indicates a precursor, the mature protein is also implied. Throughout the application, gene and protein symbols may be used interchangeably and the meaning can be derived from context as necessary.

[00616] As an illustration, a treatment can be selected for a subject suffering from Non-Small Cell Lung Cancer. One or more biomarkers, such as, but not limited to, EGFR, excision repair cross-complementation group 1 (ERCC1), p53, Ras, p27, class III beta tubulin, breast cancer gene 1 (BRCA1), breast cancer gene 1 (BRCA2), and ribonucleotide reductase messenger 1 (RRMl), can be assessed from a vesicle from the subject. Based on one or more characteristics of the one or more biomarkers, the subject can be determined to be a responder or non-responder for a treatment, such as, but not limited to, Erlotinib, Carboplatin, Paclitaxel, Gefitinib, or a combination thereof.

[00617] In another embodiment, a treatment can be selected for a subject suffering from Colorectal Cancer, and a biomarker, such as, but not limited to, K-ras, can be assessed from a vesicle from the subject. Based on one or more characteristics of the one or more biomarkers, the subject can be determined to be a responder or non-responder for a treatment, such as, but not limited to, Panitumumab, Cetuximab, or a combination thereof.

[00618] In another embodiment, a treatment can be selected for a subject suffering from Breast Cancer. One or more biomarkers, such as, but not limited to, HER2, toposiomerase II a , estrogen receptor, and progesterone receptor, can be assessed from a vesicle from the subject. Based on one or more characteristics of the one or more biomarkers, the subject can be determined to be a responder or non-responder for a treatment, such as, but not limited to, trastuzumab, anthracyclines, taxane, methotrexate, fluorouracil, or a combination thereof.

[00619] As described, the biosignature used to theranose a cancer can comprise analysis of one or more biomarker, which can be a protein or nucleic acid, including a mRNA or a microRNA. The biomarker can be detected in a bodily fluid and/or can be detected associated with a vesicle, e.g., as a vesicle antigen or as vesicle payload. In an illustrative example, the biosignature is used to identify a patient as a responder or non-responder to a tyrosine kinase inhibitor. The biomarkers can be one or more of those described in WO/2010/121238,

PREOMICS EXHIBIT 1002
Page 1112 of 1861

entitled "METHODS AND KITS TO PREDICT THERAPEUTIC OUTCOME OF TYROSINE KINASE INHIBITORS" and filed April 19, 2010; or WO/2009/105223, entitled "SYSTEMS AND METHODS OF CANCER STAGING AND TREATMENT" and filed February 19, 2009; both of which applications are incorporated herein by reference in their entirety.

[00620] In an aspect, the present invention provides a method of determining whether a subject is likely to respond or not to a tyrosine kinase inhibitor, the method comprising identifying one or more biomarker in a vesicle population in a sample from the subject, wherein differential expression of the one or more biomarker in the sample as compared to a reference indicates that the subject is a responder or non-responder to the tyrosine kinase inhibitor. In an embodiment, the one or more biomarker comprises miR-497, wherein reduced expression of miR-497 indicates that the subject is a responder (i.e., sensitive to the tyrosine kinase inhibitor). In another embodiment, the one or more biomarker comprises onr or more of miR-21, miR-23a, miR-23b, and miR-29b, wherein upregulation of the microRNA indicates that the subject is a likely non-responder (i.e., resistant to the tyrosine kinase inhibitor). In some embodiments, the one or more biomarker comprises onr or more of hsa-miR-029a, hsa-let-7d, hsa-miR-100, hsa-miR-1260, hsa-miR-025, hsa-let-7i, hsa-miR-146a, hsa-miR-594-Pre, hsa-miR-024, FGFR1, MET, RAB25, EGFR, KIT and VEGFR2. In another embodiment, the one or more biomarker comprises FGF1, HOXC10 or LHFP, wherein higher expression of the biomarker indicates that the subject is a non-responder (i.e., resistant to the tyrosine kinase inhibitor). The method can be used to determine the sensitivity of a cancer to the tyrosine kinase inhibitor, e.g., a non-small cell lung cancer cell, kidney cancer or GIST. The tyrosine kinase inhibitor can be erlotinib, vandetanib, sunitinib and/or sorafenib, or other inhibitors that operate by a similar mechanism of action. A tyrosine kinase inhibitor includes any agent that inhibits the action of one or more tyrosine kinases in a specific or non-specific fashion. Tyrosine kinase inhibitors include small molecules, antibodies, peptides, or any appropriate entity that directly, indirectly, allosterically, or in any other way inhibits tyrosine residue phosphorylation. Specific examples of tyrosine kinase inhibitors include N-(trifluoromethylphenyl)-5 -methylisoxazol-4-carboxamide, 3 - [(2,4-dimethylpyrrol-5 - yl)methylidenyl)indolin-2-one, 17-(allylamino)- 17-demethoxygeldanamycin, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-[3 -(4-morpholinyl)propoxyl] q- uinazoline, N-(3 - ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine, BIBX1382, 2,3,9, 10,1 1,12- hexahydro- lO-(hydroxymethyl)- 10-hydroxy-9-methyl-9, 12-epox- y- lH-di)ndolo[1,2,3-fg:3',2', 1 '-kl]pyrrolo[3,4-i][l,6]benzodiazocin-l-one, SH268, genistein, STI571, CEP2563, 4-(3- chlorophenylamino)-5,6-dimethyl-7H-pyrrolo[2,3-d]pyrimidinemethane sulfonate, 4-(3 -bromo- 4-hydroxyphenyl)amino-6,7-dimethoxyquinazoline, 4-(4'-hydroxyphenyl)amino-6,7-dimethoxyquinazoline, SU6668, STI571A, N-4-chlorophenyl-4-(4-pyridylmethyl)-l- phthalazinamine, N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro- 1,2- dihydro-2-oxo-3H-indol- 3-ylidine)methyl]-2,4-dimethyl-lH-pyrrole-3-carboxamide (commonly known as sunitinib), A- [A- [[4-chloro-3 (trifluoromethyl)phenyl] carbamoylamino] phenoxy] -N-methyl-pyridine-2- carboxamide (commonly known as sorafenib), EMD121974, and N-(3-ethynylphenyl)-6, 7- bis(2-methoxyethoxy)quinazolin-4-amine (commonly known as erlotinib). In some embodiments, the tyrosine kinase inhibitor has inhibitory activity upon the epidermal growth factor receptor (EGFR), VEGFR, PDGFR beta, and/or FLT3.

[00621] Thus, a treatment can be selected for the subject suffering from a cancer, based on a biosignature identified by the methods of the invention. Accordingly, the biosignature can comprise a presence or level of a circulating biomarker, including a microRNA, a vesicle, or any useful vesicle associated biomarker.

PREOMICS EXHIBIT 1002
Page 1113 of 1861

[00622] Biomarkers that can be used for theranosis of other diseases using the methods of the invention, including cardiovascular disease, neurological diseases and disorders, immune diseases and disorders and infectious disease, are described in International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein.

Biosignature Discovery

[00623] The systems and methods provided herein can be used in identifying a novel biosignature of a vesicle, such as one or more novel biomarkers for the diagnosis, prognosis or theranosis of a phenotype. In one embodiment, one or more vesicles can be isolated from a subject with a phenotype and a biosignature of the one or more vesicles determined. The biosignature can be compared to a subject without the phenotype. Differences between the two biosignatures can be determined and used to form a novel biosignature. The novel biosignature can then be used for identifying another subject as having the phenotype or not having the phenotype.

[00624] Differences between the biosignature from a subject with a particular phenotype can be compared to the biosignature from a subject without the particular phenotype. The one or more differences can be a difference in any characteristic of the vesicle. For example, the level or amount of vesicles in the sample, the half-life of the vesicle, the circulating half-life of the vesicle, the metabolic half-life of the vesicle, or the activity of the vesicle, or any combination thereof, can differ between the biosignature from the subject with a particular phenotype and the biosignature from the subject without the particular phenotype.

[00625] In some embodiments, one or more biomarkers differ between the biosignature from the subject with a particular phenotype and the biosignature from the subject without the particular phenotype. For example, the expression level, presence, absence, mutation, variant, copy number variation, truncation, duplication, modification, molecular association of one or more biomarkers, or any combination thereof, may differ between the biosignature from the subject with a particular phenotype and the biosignature from the subject without the particular phenotype. The biomarker can be any biomarker disclosed herein or that can be used to characterize a biological entity, including a circulating biomarker, such as protein or microRNA, a vesicle, or a component present in a vesicle or on the vesicle, such as any nucleic acid (e.g. RNA or DNA), protein, peptide, polypeptide, antigen, lipid, carbohydrate, or proteoglycan.

[00626] In an aspect, the invention provides a method of discovering a novel biosignature comprising comparing the biomarkers between two or more sample groups to identify biomarkers that show a difference between the sample groups. Multiple markers can be assessed in a panel format to potentially improve the performance of individual markers. In some embodiments, the multiple markers are assessed in a multiplex fashion. The ability of the individual markers and groups of markers to distinguish the groups can be assessed using statistical discriminate analysis or classification methods as used herein. Optimal panels of markers can be used as a biosignature to characterize the phenotype under analysis, such as to provide a diagnosis, prognosis or theranosis of a disease or condition. Optimization can be based on various criteria, including without limitation maximizing ROC AUC, accuracy, sensitivity at a certain specificity, or specificity at a certain sensitivity. The panels can include biomarkers from multiple types. For example, the biosignature can comprise vesicle antigens useful for capturing a vesicle population of interest, and the biosignature can further comprise payload markers within the vesicle population, including without limitation microRNAs, mRNAs, or soluble proteins. Optimal combinations can be identified as those vesicle antigens and payload markers with the greatest ROC AUC value

PREOMICS EXHIBIT 1002
Page 1114 of 1861

when comparing two settings. As another example, the biosignature can be determined by assessing a vesicle population in addition to assessing circulating biomarkers that are not obtained by isolating exosomes, such as circulating proteins and/or microRNAs.

[00627] The phenotype can be any of those listed herein, e.g., in the "Phenotype" section above. For example, the phenotype can be a proliferative disorder such as a cancer or non-malignant growth, a perinatal or pregnancy related condition, an infectious disease, a neurological disorder, a cardiovascular disease, an inflammatory disease, an immune disease, or an autoimmune disease. The cancer includes without limitation lung cancer, non-small cell lung cancerm small cell lung cancer (including small cell carcinoma (oat cell cancer), mixed small cell/large cell carcinoma, and combined small cell carcinoma), colon cancer, breast cancer, prostate cancer, liver cancer, pancreatic cancer, brain cancer, kidney cancer, ovarian cancer, stomach cancer, melanoma, bone cancer, gastric cancer, breast cancer, glioma, gliobastoma, hepatocellular carcinoma, papillary renal carcinoma, head and neck squamous cell carcinoma, leukemia, lymphoma, myeloma, or other solid tumors.

[00628] Any of the types of biomarkers or specific biomarkers described herein can be assessed as part of a biosignature. Exemplary biomarkers include without limitation those in **Tables 3** and **5.** The markers in the tables can be used for capture and/or detection of vesicles for characterizing phenotypes as disclosed herein. In some cases, multiple capture and/or detectors are used to enhance the characterization. The markers can be detected as protein or as mRNA, which can be circulating freely or in complex. The markers can be detected as vesicle surface antigens or and vesicle payload. The "Illustrate Class" indicates indications for which the markers are known markers. Those of skill will appreciate that the markers can also be used in alternate settings in certain instances. For example, a marker which can be used to characterize one type disease may also be used to characterize another disease.

[00629] Any of the types of biomarkers or specific biomarkers described herein can be assessed to discover a novel biosignature, e.g., the biomarkers in **Tables 3-5.** In an embodiment, the biomarkers selected for discovery comprise cell-specific biomarkers as listed herein, including without limitation the genes and microRNA listed in **Figs. 1-60** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein, **Tables 9-10** or **Table 16.** The biomarkers can comprise one or more disease associated, drug associated, or prognostic target such as listed in **Table 11.** The biomarkers can comprise one or more general vesicle marker, one or more cell-specific vesicle marker, and/or one or more disease-specific vesicle marker.

Table 11: Disease- and Drug-associated Biomarkers

Gene Symbol	Gene Name	Protein Symbol	Protein Name
ABCB1, PGP	ATP-binding cassette, sub-family B (MDR/TAP), member 1	ABCB1, MDR1, PGP	Multidrug resistance protein 1; P-glycoprotein
ABCC1, MRP1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	MRP1, ABCC1	Multidrug resistance-associated protein 1
ABCG2, BCRP	ATP-binding cassette, sub-family G (WHITE), member 2	ABCG2	ATP-binding cassette sub-family G member 2
ACE2	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	ACE2	Angiotensin-converting enzyme 2 precursor
ADA	adenosine deaminase	ADA	Adenosine deaminase
ADH1C	alcohol dehydrogenase 1C (class I), gamma polypeptide	ADH1G	Alcohol dehydrogenase 1C
ADH4	alcohol dehydrogenase 4 (class II), pi polypeptide	ADH4	Alcohol dehydrogenase 4

PREOMICS EXHIBIT 1002
Page 1115 of 1861

AGT	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	ANGT, AGT	Angiotensinogen precursor
ALK	anaplastic lymphoma receptor tyrosine kinase	ALK	ALK tyrosine kinase receptor precursor
AR	androgen receptor	AR	Androgen receptor
AREG	amphiregulin	AREG	Amphiregulin precursor
ASNS	asparagine synthetase	ASNS	Asparagine synthetase [glutamine-hydrolyzing]
BCL2	B-cell CLL/lymphoma 2	BCL2	Apoptosis regulator Bcl-2
BDCA1, CD1C	CD1c molecule	CD1C	T-cell surface glycoprotein CD1c precursor
BIRC5	baculoviral IAP repeat-containing 5	BIRC5, Survivin	Baculoviral IAP repeat-containing protein 5; Survivin
BRAF	v-raf murine sarcoma viral oncogene homolog B1	B-RAF, BRAF	Serine/threonine-protein kinase B-raf
BRCA1	breast cancer 1, early onset	BRCA1	Breast cancer type 1 susceptibility protein
BRCA2	breast cancer 2, early onset	BRCA2	Breast cancer type 2 susceptibility protein
CA2	carbonic anhydrase II	CA2	Carbonic anhydrase 2
CAV1	caveolin 1, caveolae protein, 22kDa	CAV1	Caveolin-1
CCND1	cyclin D1	CCND1, Cyclin D1, BCL-1	G1/S-specific cyclin-D1
CD20, MS4A1	membrane-spanning 4-domains, subfamily A, member 1	CD20	B-lymphocyte antigen CD20
CD25, IL2RA	interleukin 2 receptor, alpha	CD25	Interleukin-2 receptor subunit alpha precursor
CD33	CD33 molecule	CD33	Myeloid cell surface antigen CD33 precursor
CD52, CDW52	CD52 molecule	CD52	CAMPATH-1 antigen precursor
CDA	cytidine deaminase	CDA	Cytidine deaminase
CDH1, ECAD	cadherin 1, type 1, E-cadherin (epithelial)	E-Cad	Cadherin-1 precursor (E-cadherin)
CDK2	cyclin-dependent kinase 2	CDK2	Cell division protein kinase 2
CDKN1A, P21	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A, p21	Cyclin-dependent kinase inhibitor 1
CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	CDKN1B, p27	Cyclin-dependent kinase inhibitor 1B
CDKN2A, P16	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	CD21A, p16	Cyclin-dependent kinase inhibitor 2A, isoforms 1/2/3
CES2	carboxylesterase 2 (intestine, liver)	CES2, EST2	Carboxylesterase 2 precursor
CK 5/6	cytokeratin 5 / cytokeratin 6	CK 5/6	Keratin, type II cytoskeletal 5; Keratin, type II cytoskeletal 6
CK14, KRT14	keratin 14	CK14	Keratin, type I cytoskeletal 14
CK17, KRT17	keratin 17	CK17	Keratin, type I cytoskeletal 17
COX2, PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	COX-2, PTGS2	Prostaglandin G/H synthase 2 precursor
DCK	deoxycytidine kinase	DCK	Deoxycytidine kinase
DHFR	dihydrofolate reductase	DHFR	Dihydrofolate reductase
DNMT1	DNA (cytosine-5-)-methyltransferase 1	DNMT1	DNA (cytosine-5)-methyltransferase 1
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	DNMT3A	DNA (cytosine-5)-methyltransferase 3A
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	DNMT3B	DNA (cytosine-5)-methyltransferase 3B
ECGF1, TYMP	thymidine phosphorylase	TYMP, PD-ECGF,	Thymidine phosphorylase precursor

PREOMICS EXHIBIT 1002
Page 1116 of 1861

		ECDF1	
EGFR, ERBB1, HER1	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	EGFR, ERBB1, HER1	Epidermal growth factor receptor precursor
EML4	echinoderm microtubule associated protein like 4	EML4	Echinoderm microtubule-associated protein-like 4
EPHA2	EPH receptor A2	EPHA2	Ephrin type-A receptor 2 precursor
ER, ESR1	estrogen receptor 1	ER, ESR1	Estrogen receptor
ERBB2, HER2/NEU	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	ERBB2, HER2, HER-2/neu	Receptor tyrosine-protein kinase erbB-2 precursor
ERCC1	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	ERCC1	DNA excision repair protein ERCC-1
ERCC3	excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complementing)	ERCC3	TFIIH basal transcription factor complex helicase XPB subunit
EREG	Epiregulin	EREG	Proepiregulin precursor
FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	FLT-1, VEGFR1	Vascular endothelial growth factor receptor 1 precursor
FOLR1	folate receptor 1 (adult)	FOLR1	Folate receptor alpha precursor
FOLR2	folate receptor 2 (fetal)	FOLR2	Folate receptor beta precursor
FSHB	follicle stimulating hormone, beta polypeptide	FSHB	Follitropin subunit beta precursor
FSHPRH1, CENP1	centromere protein I	FSHPRH1, CENP1	Centromere protein I
FSHR	follicle stimulating hormone receptor	FSHR	Follicle-stimulating hormone receptor precursor
FYN	FYN oncogene related to SRC, FGR, YES	FYN	Tyrosine-protein kinase Fyn
GART	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	GART, PUR2	Trifunctional purine biosynthetic protein adenosine-3
GNA11, GA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	GNA11, G alpha-11, G-protein subunit alpha-11	Guanine nucleotide-binding protein subunit alpha-11
GNAQ, GAQ	guanine nucleotide binding protein (G protein), q polypeptide	GNAQ	Guanine nucleotide-binding protein G(q) subunit alpha
GNRH1	gonadotropin-releasing hormone 1 (luteinizing-releasing hormone)	GNRH1, GON1	Progonadoliberin-1 precursor
GNRHR1, GNRHR	gonadotropin-releasing hormone receptor	GNRHR1	Gonadotropin-releasing hormone receptor
GSTP1	glutathione S-transferase pi 1	GSTP1	Glutathione S-transferase P
HCK	hemopoietic cell kinase	HCK	Tyrosine-protein kinase HCK
HDAC1	histone deacetylase 1	HDAC1	Histone deacetylase 1
HGF	hepatocyte growth factor (hepapoietin A; scatter factor)	HGF	Hepatocyte growth factor precursor
HIF1A	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	HIF1A	Hypoxia-inducible factor 1-alpha
HIG1, HIGD1A,	HIG1 hypoxia inducible domain family, member 1A	HIG1, HIGD1A,	HIG1 domain family member 1A

PREOMICS EXHIBIT 1002
Page 1117 of 1861

HIG1A		HIG1A	
HSP90AA1, HSP90, HSPCA	heat shock protein 90kDa alpha (cytosolic), class A member 1	HSP90, HSP90A	Heat shock protein HSP 90-alpha
IGF1R	insulin-like growth factor 1 receptor	IGF-1R	Insulin-like growth factor 1 receptor precursor
IGFBP3, IGFRBP3	insulin-like growth factor binding protein 3	IGFBP-3, IBP-3	Insulin-like growth factor-binding protein 3 precursor
IGFBP4, IGFRBP4	insulin-like growth factor binding protein 4	IGFBP-4, IBP-4	Insulin-like growth factor-binding protein 4 precursor
IGFBP5, IGFRBP5	insulin-like growth factor binding protein 5	IGFBP-5, IBP-5	Insulin-like growth factor-binding protein 5 precursor
IL13RA1	interleukin 13 receptor, alpha 1	IL-13RA1	Interleukin-13 receptor subunit alpha-1 precursor
KDR	kinase insert domain receptor (a type III receptor tyrosine kinase)	KDR, VEGFR2	Vascular endothelial growth factor receptor 2 precursor
KIT, c-KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	KIT, c-KIT, CD117, SCFR	Mast/stem cell growth factor receptor precursor
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	K-RAS	GTPase KRas precursor
LCK	lymphocyte-specific protein tyrosine kinase	LCK	Tyrosine-protein kinase Lck
LTB	lymphotoxin beta (TNF superfamily, member 3)	LTB, TNF3	Lymphotoxin-beta
LTBR	lymphotoxin beta receptor (TNFR superfamily, member 3)	LTBR, LTBR3, TNFR	Tumor necrosis factor receptor superfamily member 3 precursor
LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	LYN	Tyrosine-protein kinase Lyn
MET, c-MET	met proto-oncogene (hepatocyte growth factor receptor)	MET, c-MET	Hepatocyte growth factor receptor precursor
MGMT	O-6-methylguanine-DNA methyltransferase	MGMT	Methylated-DNA--protein-cysteine methyltransferase
MKI67, KI67	antigen identified by monoclonal antibody Ki-67	Ki67, Ki-67	Antigen KI-67
MLH1	mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)	MLH1	DNA mismatch repair protein Mlh1
MMR	mismatch repair (refers to MLH1, MSH2, MSH5)		
MSH2	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	MSH2	DNA mismatch repair protein Msh2
MSH5	mutS homolog 5 (E. coli)	MSH5, hMSH5	MutS protein homolog 5
MYC, c-MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	MYC, c-MYC	Myc proto-oncogene protein
NBN, P95	nibrin	NBN, p95	Nibrin
NDGR1	N-myc downstream regulated 1	NDGR1	Protein NDGR1
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	NFKB1	Nuclear factor NF-kappa-B p105 subunit
NFKB2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	NFKB2	Nuclear factor NF-kappa-B p100 subunit
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	NFKBIA	NF-kappa-B inhibitor alpha
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	NRAS	GTPase NRas, Transforming protein N-Ras
ODC1	ornithine decarboxylase 1	ODC	Ornithine decarboxylase
OGFR	opioid growth factor receptor	OGFR	Opioid growth factor receptor
PARP1	poly (ADP-ribose) polymerase 1	PARP-1	Poly [ADP-ribose] polymerase 1
PDGFC	platelet derived growth factor C	PDGF-C,	Platelet-derived growth factor C precursor

PREOMICS EXHIBIT 1002
Page 1118 of 1861

		VEGF-E	
PDGFR	platelet-derived growth factor receptor	PDGFR	Platelet-derived growth factor receptor
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	PDGFRA, PDGFR2, CD140 A	Alpha-type platelet-derived growth factor receptor precursor
PDGFRB	platelet-derived growth factor receptor, beta polypeptide	PDGFRB, PDGFR, PDGFR1, CD140 B	Beta-type platelet-derived growth factor receptor precursor
PGR	progesterone receptor	PR	Progesterone receptor
PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide	PI3K subunit p110α	phosphoinositide-3-kinase, catalytic, alpha polypeptide
POLA1	polymerase (DNA directed), alpha 1, catalytic subunit; polymerase (DNA directed), alpha, polymerase (DNA directed), alpha 1	POLA, POLA1, p180	DNA polymerase alpha catalytic subunit
PPARG, PPARG1, PPARG2, PPAR-gamma, NR1C3	peroxisome proliferator-activated receptor gamma	PPARG	Peroxisome proliferator-activated receptor gamma
PPARGC1A, LEM6, PGC1, PGC1A, PPARGC1	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	PGC-1-alpha, PPARGC-1-alpha	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPAR-gamma coactivator 1-alpha
PSMD9, P27	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	p27	26S proteasome non-ATPase regulatory subunit 9
PTEN, MMAC1, TEP1	phosphatase and tensin homolog	PTEN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase; Mutated in multiple advanced cancers 1
PTPN12	protein tyrosine phosphatase, non-receptor type 12	PTPG1	Tyrosine-protein phosphatase non-receptor type 12; Protein-tyrosine phosphatase G1
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	RAF, RAF-1, c-RAF	RAF proto-oncogene serine/threonine-protein kinase
RARA	retinoic acid receptor, alpha	RAR, RAR-alpha, RARA	Retinoic acid receptor alpha
ROS1, ROS, MCF3	c-ros oncogene 1, receptor tyrosine kinase	ROS1, ROS	Proto-oncogene tyrosine-protein kinase ROS
RRM1	ribonucleotide reductase M1	RRM1, RR1	Ribonucleoside-diphosphate reductase large subunit
RRM2	ribonucleotide reductase M2	RRM2, RR2M, RR2	Ribonucleoside-diphosphate reductase subunit M2
RRM2B	ribonucleotide reductase M2 B (TP53 inducible)	RRM2B, P53R2	Ribonucleoside-diphosphate reductase subunit M2 B
RXRB	retinoid X receptor, beta	RXRB	Retinoic acid receptor RXR-beta
RXRG	retinoid X receptor, gamma	RXRG, RXRC	Retinoic acid receptor RXR-gamma
SIK2	salt-inducible kinase 2	SIK2, Q9H0K1	Salt-inducible protein kinase 2; Serine/threonine-protein kinase SIK2
SLC29A1	solute carrier family 29 (nucleoside transporters), member 1	ENT-1	Equilibrative nucleoside transporter 1
SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	SPARC	SPARC precursor; Osteonectin
SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	SRC	Proto-oncogene tyrosine-protein kinase Src
SSTR1	somatostatin receptor 1	SSTR1,	Somatostatin receptor type 1

PREOMICS EXHIBIT 1002
Page 1119 of 1861

		SSR1, SS1R	
SSTR2	somatostatin receptor 2	SSTR2, SSR2, SS2R	Somatostatin receptor type 2
SSTR3	somatostatin receptor 3	SSTR3, SSR3, SS3R	Somatostatin receptor type 3
SSTR4	somatostatin receptor 4	SSTR4, SSR4, SS4R	Somatostatin receptor type 4
SSTR5	somatostatin receptor 5	SSTR5, SSR5, SS5R	Somatostatin receptor type 5
TK1	thymidine kinase 1, soluble	TK1, KITH	Thymidine kinase, cytosolic
TLE3	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	TLE3	Transducin-like enhancer protein 3
TNF	tumor necrosis factor (TNF superfamily, member 2)	TNF, TNF-alpha, TNF-a	Tumor necrosis factor precursor
TOP1, TOPO1	topoisomerase (DNA) I	TOP1, TOPO1	DNA topoisomerase 1
TOP2A, TOPO2A	topoisomerase (DNA) II alpha 170kDa	TOP2A, TOP2, TOPO2A	DNA topoisomerase 2-alpha; Topoisomerase II alpha
TOP2B, TOPO2B	topoisomerase (DNA) II beta 180kDa	TOP2B, TOPO2B	DNA topoisomerase 2-beta; Topoisomerase II beta
TP53	tumor protein p53	p53	Cellular tumor antigen p53
TUBB3	tubulin, beta 3	Beta III tubulin, TUBB3, TUBB4	Tubulin beta-3 chain
TXN	thioredoxin	TXN, TRX, TRX-1	Thioredoxin
TXNRD1	thioredoxin reductase 1	TXNRD1, TXNR	Thioredoxin reductase 1, cytoplasmic; Oxidoreductase
TYMS, TS	thymidylate synthetase	TYMS, TS	Thymidylate synthase
VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	VDR	Vitamin D3 receptor
VEGFA, VEGF	vascular endothelial growth factor A	VEGF-A, VEGF	Vascular endothelial growth factor A precursor
VEGFC	vascular endothelial growth factor C	VEGF-C	Vascular endothelial growth factor C precursor
VHL	von Hippel-Lindau tumor suppressor	VHL	Von Hippel-Lindau disease tumor suppressor
YES1	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	YES1, Yes, p61-Yes	Proto-oncogene tyrosine-protein kinase Yes
ZAP70	zeta-chain (TCR) associated protein kinase 70kDa	ZAP-70	Tyrosine-protein kinase ZAP-70

[00630] The biomarkers used for biosignature discovery can comprise include markers commonly associated with vesicles, including without limitation one or more vesicle biomarker in **Table 3** or **5.** Other biomarkers can be selected from those disclosed in the ExoCarta database, available at exocarta.ludwig.edu.au, which discloses proteins and RNA molecules identified in vesicles. See also Mathivanan and Simpson, ExoCarta: A compendium of exosomal proteins and RNA. Proteomics. 2009 Nov 9(21):4997-5000.

[00631] The biomarkers used for biosignature discovery can comprise include markers commonly associated with vesicles, including without limitation one or more of A33, a33 nl5, AFP, ALA, ALIX, ALP, AnnexinV, APC, ASCA, ASPH (246-260), ASPH (666-680), ASPH (A-10), ASPH (D01P), ASPH (D03), ASPH (G-20), ASPH (H-300), AURKA, AURKB, B7H3, B7H4, BCA-225, BCNP, BCNP1, BDNF, BRCA, CA125 (MUC16), CA-19-9, C-Bir, CD1 .1, CD10, CD174 (Lewis y), CD24, CD44, CD46, CD59 (MEM-43), CD63,

PREOMICS EXHIBIT 1002
Page 1120 of 1861

CD66e CEA, CD73, CD81, CD9, CDA, CDAC1 la2, CEA, C-Erb2, C-erbB2, CRMP-2, CRP, CXCL12, CYFRA21-1, DLL4, DR3, EGFR, Epcam, EphA2, EphA2 (H-77), ER, ErbB4, EZH2, FASL, FRT, FRT c.f23, GDF15, GPCR, GPR30, Gro-alpha, HAP, HBD 1, HBD2, HER 3 (ErbB3), HSP, HSP70, hVEGFR2, iC3b, IL 6 Unc, IL-1B, IL6 Unc, IL6R, IL8, IL-8, INSIG-2, KLK2, LICAM, LAMN, LDH, MACC-1, MAPK4, MART-1, MCP-1, M-CSF, MFG-E8, MICl, MIF, MIS RII, MMG, MMP26, MMP7, MMP9, MS4A1, MUC1, MUC1 seql, MUC1 seql 1A, MUC17, MUC2, Ncam, NGAL, NPGP/NPFF2, OPG, OPN, p53, p53, PA2G4, PBP, PCSA, PDGFRB, PGP9.5, PIM1, PR (B), PRL, PSA, PSMA, PSME3, PTEN, R5-CD9 Tube 1, Reg IV, RUNX2, SCRNl, seprase, SERPINB3, SPARC, SPB, SPDEF, SRVN, STAT 3, STEAP1, TF (FL-295), TFF3, TGM2, TIMP-1, TIMP1, TIMP2, TMEM21 1, TMPRSS2, TNF-alpha, Trail-R2, Trail-R4, TrKB, TROP2, Tsg 101, TWEAK, UNC93A, VEGF A, and YPSMA-1 . The biomarkers can include one or more of NSE, TRIM29, CD63, CD15 1, ASPH, LAMP2, TSPAN1, SNAIL, CD45, CKS1, NSE, FSHR, OPN, FTH1, PGP9, ANNEXIN 1, SPD, CD81, EPCAM, PTH1R, CEA, CYTO 7, CCL2, SPA, KRAS, TWIST 1, AURKB, MMP9, P27, MMP1, HLA, HIF, CEACAM, CENPH, BTUB, INTO b4, EGFR, NACC1, CYTO 18, NAP2, CYTO 19, ANNEXIN V, TGM2, ERB2, BRCA1, B7H3, SFTPC, PNT, NCAM, MS4A1, P53, INGA3, MUC2, SPA, OPN, CD63, CD9, MUC1, UNCR3, PAN ADH, HCG, TIMP, PSMA, GPCR, RACK1, PCSA, VEGF, BMP2, CD81, CRP, PRO GRP, B7H3, MUC1, M2PK, CD9, PCSA, and PSMA. The biomarkers can also include one or more of TFF3, MS4A1, EphA2, GAL3, EGFR, N-gal, PCSA, CD63, MUC1, TGM2, CD81, DR3, MACC-1, TrKB, CD24, TIMP-1, A33, CD66 CEA, PRL, MMP9, MMP7, TMEM21 1, SCRNl, TROP2, TWEAK, CDACC1, UNC93A, APC, C-Erb, CD10, BDNF, FRT, GPR30, P53, SPR, OPN, MUC2, GRO-1, tsg 101 and GDF15. In embodiments, the biomarkers used to discover a biosignature comprise one or more of those shown in **Figs. 99, 100, 108A-C, 114A,** and/or **115A-E** of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein.

[00632] The markers can include one or more of NY-ESO-1, SSX-2, SSX-4, XAGE-lb, AMACR, p90 autoantigen, LEDGF. See Xie et al., Journal of Translational Medicine 201 1, 9:43, which publication is incorporated by reference in its entirety herein. The markers can include one or more of STEAP and EZH2. See Hayashi et al., Journal of Translational Medicine 201 1, 9:191, which publication is incorporated by reference in its entirety herein. The markers can include one or more members of the miR-1 83-96-182 cluster (miRs-183, 96 and 182, which are expressed as a cluster and share sequence similarity) or a zinc transporter, such as hZIPl. See Mihelich et al., The miR-1 83-96-1 82 cluster is overexpressed in prostate tissue and regulates zinc homeostasis in prostate cells. J Biol Chem. 201 1 Nov 1. [Epub ahead of print], which publication is incorporated by reference in its entirety herein.

[00633] The markers can include one or more of RAD23B, FBP1, TNFRSF1A, CCNG2, NOTCH3, ETV1, BID, SIM2, LETMD1, ANXA1, miR-5 19d, and miR-647. The markers can include one or more of RAD23B, FBP1, TNFRSF 1A, NOTCH3, ETV1, BID, SIM2, ANXAl and BCL2. See Long et al., Am J Pathol. 201 1 Jul;179(l):46-54, which publication is incorporated by reference in its entirety herein. The markers can include one or more of ANPEP, ABLl, PSCA, EFNAl, HSPBl, INMT and TRIP13. See Larkin et al, British Journal of Cancer (201 1), 1 -9. These markers can be assessed as RNA or protein. In an embodiment, one or more of these markers are used predict recurrence or prostate cancer. In another embodiment, ANPEP and ABLl or ANPEP and PSCA are assessed to predict aggressiveness.

PREOMICS EXHIBIT 1002
Page 1121 of 1861

[00634] One of skill will appreciate that any marker disclosed herein or that can be compared between two samples or sample groups of interest can be used to discover a novel biosignature for any given biological setting that can be compared, e.g., healthy versus diseased, late stage versus early stage disease, drug responder versus non-responder, disease 1 versus disease 2, and the like. Markers, such as one or more marker disclosed herein such as in **Tables 5** or **11,** can then be chosen individually or as a panel to form a biosignature that can be used to characterize the phenotype.

[00635] The one or more differences can then be used to form a candidate biosignature for the particular phenotype, such as the diagnosis of a condition, diagnosis of a stage of a disease or condition, prognosis of a condition, or theranosis of a condition. The novel biosignature can then be used to identify the phenotype in other subjects. The biosignature of a vesicle for a new subject can be determined and compared to the novel signature to determine if the subject has the particular phenotype for which the novel biosignature was identified from.

[00636] For example, the biosignature of a subject with cancer can be compared to another subject without cancer. Any differences can be used to form a novel biosignature for the diagnosis of the cancer. In another embodiment, the biosignature of a subject with an advanced stage of cancer can be compared to another subject with a less advanced stage of cancer. Any differences can be used to form a novel biosignature for the classification of the stage of cancer. In yet another embodiment, the biosignature of a subject with an advanced stage of cancer can be compared to another subject with a less advanced stage of cancer. Any differences can be used to form a novel biosignature for the classification of the stage of cancer.

[00637] In one embodiment, the phenotype is drug resistance or non-responsiveness to a therapeutic. One or more vesicles can be isolated from a non-responder to a particular treatment and the biosignature of the vesicle determined. The biosignature of the vesicle obtained from the non-responsder can be compared to the biosignature of a vesicle obtained from a responsder. Differences between the biosignature from the non-responder can be compared to the biosignature from the responder. The one or more differences can be a difference in any characteristic of the vesicle. For example, the level or amount of vesicles in the sample, the half-life of the vesicle, the circulating half-life of the vesicle, the metabolic half-life of the vesicle, the activity of the vesicle, or any combination thereof, can differ between the biosignature from the non-responder and the biosignature from the responder.

[00638] In some embodiments, one or more biomarkers differ between the biosignature from the non-responder and the biosignature from the responder. For example, the expression level, presence, absence, mutation, variant, copy number variation, truncation, duplication, modification, molecular association of one or more biomarkers, or any combination thereof, may differ between the biosignature from the non-responder and the biosignature from the responder.

[00639] In some embodiments, the difference can be in the amount of drug or drug metabolite present in the vesicle. Both the responder and non-responder can be treated with a therapeutic. A comparison between the biosignature from the responder and the biosignature from the non-responder can be performed, the amount of drug or drug metabolite present in the vesicle from the responder differs from the amount of drug or drug metabolite present in the non-responder. The difference can also be in the half-life of the drug or drug metabolite. A difference in the amount or half-life of the drug or drug metabolite can be used to form a novel biosignature for identifying non-responders and responders.

PREOMICS EXHIBIT 1002
Page 1122 of 1861

[00640] A vesicle useful for methods and compositions described herein can be discovered by taking advantage of its physicochemical characteristics. For example, a vesicle can be discovered by its size, e.g., by filtering biological matter in a known range from 30 - 120 nm in diameter. Size-based discovery methods, such as differential centrifugation, sucrose gradient centrifugation, or filtration have been used for isolation of a vesicle.

[00641] A vesicle can be discovered by its molecular components. Molecular property-based discovery methods include, but are not limited to, immunological isolation using antibodies recognizing molecules associated with vesicle. For example, a surface molecule associated with a vesicle includes, but not limited to, a MHC-II molecule, CD63, CD81, LAMP-1, Rab7 or Rab5.

[00642] Various techniques known in the art are applicable for validation and characterization of a vesicle. Techniques useful for validation and characterization of a vesicle includes, but is not limited to, western blot, electron microscopy, immunohistochemistry, immunoelectron microscopy, FACS (Fluorescent activated cell sorting), electrophoresis (1 dimension, 2 dimension), liquid chromatography, mass spectrometry, MALDI-TOF (matrix assisted laser desorption/ionization-time of flight), ELISA, LC-MS-MS, and nESI (nanoelectrospray ionization). For example U.S. Pat. No. 2009/0148460 describes use of an ELISA method to characterize a vesicle. U.S. Pat. No. 2009/0258379 describes isolation of membrane vesicles from biological fluids.

[00643] Vesicles can be further analyzed for one or more nucleic acids, lipids, proteins or polypeptides, such as surface proteins or peptides, or proteins or peptides within a vesicle. Candidate peptides can be identified by various techniques including mass spectrometry coupled with purification methods such as liquid chromatography. A peptide can then be isolated and its sequence can be identified by sequencing. A computer program that predicts a sequence based on exact mass of a peptide can also be used to reveal the sequence of a peptide isolated from a vesicle. For example, LTQ-Orbitrap mass spectrometry can be used for high sensitivity and high accuracy peptide sequencing. LTQ-Orbitrap method has been described (Simpson et al, Expert Rev. Proteomics 6:267-283, 2009), which is incorporated herein by reference in its entirety.

Vesicle Compositions

[00644] Also provided herein is an isolated vesicle with a particular biosignature. The isolated vesicle can comprise one or more biomarkers or biosignatures specific for specific cell type, or for characterizing a phenotype, such as described above. An isolated vesicle can also comprise one or more biomarkers, wherein the expression level of the one or more biomarkers is higher, lower, or the same for an isolated vesicle as compared to an isolated vesicle derived from a normal cell (ie. a cell derived from a subject without a phenotype of interest). For example, an isolated vesicle can comprise one or more biomarkers selected from **Table 5.** In an embodiment, the one or more biomarkers are selected from the group consisting of: B7H3, PSCA, MFG-E8, Rab, STEAP, PSMA, PCSA, 5T4, miR-9, miR-629, miR- 141, miR-671-3p, miR-491, miR-1 82, miR-125a-3p, miR-324-5p, miR-148b, and miR-222, wherein the expression level of the one or more biomarkers is higher for an isolated vesicle as compared those derived from a normal cell. The biomarkers can comprise one or more of ADAM- 10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2 and SSX4. For example, the biomarkers can be one or more of EGFR, EpCAM, KLK2, PBP, SPDEF, SSX2 and SSX4. The isolated vesicle can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, or 19 of the biomarkers selected from the group. The isolated vesicle can further comprising one or more biomarkers selected from the group consisting of: EpCam, B7H3, PSMA, PSCA, PCSA, CD63, CD59, CD81, or CD9. The

PREOMICS EXHIBIT 1002
Page 1123 of 1861

isolated vesicles can be PCSA+, Muc2+, Adaml0+ vesicles. The isolated vesicles can be MMP7+ vesicles. The isolated vesicles can be Ago+ vesicles.

[00645] A composition comprising an isolated vesicle is also provided herein. The composition can comprise one or more isolated vesicles. For example, the composition can comprise a plurality of vesicles, or one or more populations of vesicles. The composition can be substantially enriched for vesicles. For example, the composition can be substantially absent of cellular debris, cells, or non-exosomal proteins, peptides, or nucleic acids (such as biological molecules not contained within the vesicles). The cellular debris, cells, or non-exosomal proteins, peptides, or nucleic acids, can be present in a biological sample along with vesicles. A composition can be substantially absent of cellular debris, cells, or non-exosomal proteins, peptides, or nucleic acids (such as biological molecules not contained within the vesicles), can be obtained by any method disclosed herein, such as through the use of one or more binding agents or capture agents for one or more vesicles. The vesicles can comprise at least 30, 40, 50, 60, 70, 80, 90, 95 or 99% of the total composition, by weight or by mass. The vesicles of the composition can be a heterogeneous or homogeneous population of vesicles. For example, a homogeneous population of vesicles comprises vesicles that are homogeneous as to one or more properties or characteristics. For example, the one or more characteristics can be selected from a group consisting of: one or more of the same biomarkers, a substantially similar or identical biosignature, derived from the same cell type, vesicles of a particular size, and a combination thereof.

[00646] Thus, in some embodiments, the composition comprises a substantially enriched population of vesicles. The composition can be enriched for a population of vesicles that are at least 30, 40, 50, 60, 70, 80, 90, 95 or 99% homogeneous as to one or more properties or characteristics. For example, the one or more characteristics can be selected from a group consisting of: one or more of the same biomarkers, a substantially similar or identical biosignature, derived from the same cell type, vesicles of a particular size, and a combination thereof. For example, the population of vesicles can be homogeneous by all having a particular biosignature, having the same biomarker, having the same biomarker combination, or derived from the same cell type. In some embodients, the composition comprises a substantially homogeneous population of vesicles, such as a population with a specific biosignature, derived from a specific cell, or both.

[00647] The population of vesicles can comprise one or more of the same biomarkers. The biomarker can be any component such as any nucleic acid (e.g. RNA or DNA), protein, peptide, polypeptide, antigen, lipid, carbohydrate, or proteoglycan. For example, each vesicle in a population can comprise the same or identical one or more biomarkers. In some embodiments, each vesicle comprises the same 1, 2, 3, 4, 5, 6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 biomarkers.

[00648] The vesicle population comprising the same or identical biomarker can include each vesicle in the population having the same presence or absence, expression level, mutational state, or modification of the biomarker. For example, an enriched population of vesicle can comprise vesicles wherein each vesicle has the same biomarker present, the same biomarker absent, the same expression level of a biomarker, the same modification of a biomarker, or the same mutation of a biomarker. The same expression level of a biomarker can refer to a quantitative or qualitive measurement, such as the vesicles in the population underexpress, overexpress, or have the same expression level of a biomarker as compared to a reference level.

[00649] Alternatively, the same expression level of a biomarker can be a numerical value representing the expression of a biomarker that is similar for each vesicle in a population. For example the copy number of a

PREOMICS EXHIBIT 1002
Page 1124 of 1861

miRNA, the amount of protein, or the level of mRNA of each vesicle, can be quantitatively similar for each vesicle in a population, such that the numerical amount of each vesicle is ±1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20% from the amount in each other vesicle in the population, as such variations are appropriate.

[00650] In some embodiments, the composition comprises a substantially enriched population of vesicles, wherein the vesicles in the enriched population has a substantially similar or identical biosignature. The biosignature can comprise one or more characteristic of the vesicle, such as the level or amount of vesicles, temporal evaluation of the variation in vesicle half-life, circulating vesicle half-life, metabolic half-life of a vesicle, or the activity of a vesicle. The biosignature can also comprise the presence or absence, expression level, mutational state, or modification of a biomarker, such as those described herein.

[00651] The biosignature of each vesicle in the population can be at least 30, 40, 50, 60, 70, 80, 90, 95, or 99% identical. In some embodiments, the biosignature of each vesicle is 100% identical. The biosignature of each vesicle in the enriched population can have the same 1, 2, 3, 4, 5, 6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 characteristics. For example, a biosignature of a vesicle in an enriched population can be the presence of a first biomarker, the presence of a second biomarker, and the underexpression of a third biomarker. Another vesicle in the same population can be 100% identical, having the same first and second biomarkers present and underexpression of the third biomarker. Alternatively, a vesicle in the same population can have the same first and second biomarkers, but not have underexpression of the third biomarker.

[00652] In some embodiments, the composition comprises a substantially enriched population of vesicles, wherein the vesicles are derived from the same cell type. For example, the vesicles can all be derived from cells of a specific tissue, cells from a specific tumor of interest or a diseased tissue of interest, circulating tumor cells, or cells of maternal or fetal origin. The vesicles can all be derived from tumor cells. The vesicles can all be derived from the same tissue or cells, including without limitation lung, pancreas, stomach, intestine, bladder, kidney, ovary, testis, skin, colorectal, breast, prostate, brain, esophagus, liver, placenta, or fetal cells.

[00653] The composition comprising a substantially enriched population of vesicles can also comprise vesicles are of a particular size. For example, the vesicles can all a diameter of greater than about 10, 20, or 30nm. They can all have a diameter of about 10-lOOOnm, e.g., about 30-800nm, about 30-200nm, or about 30-100nm. In some embodiments, the vesicles can all have a diameter of less than 10,000nm, lOOOnm, 800nm, 500nm, 200nm, lOOnm or 50 nm.

[00654] The population of vesicles homogeneous for one or more characteristics can comprises at least about 30, 40, 50, 60, 70, 80, 90, 95, or 99% of the total vesicle population of the composition. In some embodiments, a composition comprising a substantially enriched population of vesicles comprises at least 2, 3, 4, 5, 10, 20, 25, 50, 100, 250, 500, or 1000 times the concentration of vesicle as compared to a concentration of the vesicle in a biological sample from which the composition was derived. In yet other embodiments, the composition can further comprise a second enriched population of vesicles, wherein the poplulation of vesicles is at least 30% homogeneous as to one or more characteristics, as described herein.

[00655] Multiplex analysis can be used to obtain a composition substantially enriched for more than one population of vesicles, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10 vesicle, populations. Each substantially enriched vesicle population can comprise at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 46, 47, 48, or 49% of the composition,

PREOMICS EXHIBIT 1002
Page 1125 of 1861

by weight or by mass. In some embodiments, the substantially enriched vesicle population comprises at least about 30, 40, 50, 60, 70, 80, 90, 95, or 99% of the composition, by weight or by mass.

[00656] A substantially enriched population of vesicles can be obtained by using one or more methods, processes, or systems as disclosed herein. For example, isolation of a population of vesicles from a sample can be performed by using one or more binding agents for one or more biomarkers of a vesicle, such as using two or more binding agents that target two or more biomarkers of a vesicle. One or more capture agents can be used to obtain a substantially enriched population of vesicles. One or more detection agents can be used to identify a substantially enriched population of vesicles.

[00657] In one embodiment, a population of vesicles with a particular biosignature is obtained by using one or more binding agents for the biomarkers of the biosignature. The vesicles can be isolated resulting in a composition comprising a substantially enriched population of vesicles with the particular biosignature. In another embodiment, a population of vesicles with a particular biosignature of interest can be obtained by using one or more binding agents for biomarkers that are not a component of the biosignature of interest. Thus, the binding agents can be used to remove the vesicles that do not have the biosignature of interest and the resulting composition is substantially enriched for the population of vesicles with the particular biosignature of interest. The resulting composition can be substantially absent of the vesicles comprising a biomarker for the binding agent.

[00658] International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein.

Detection System and Kits

[00659] Also provided is a detection system configured to determine one or more biosignatures for a vesicle. The detection system can be used to detect a heterogeneous population of vesicles or one or more homogeneous population of vesicles. The detection system can be configured to detect a plurality of vesicles, wherein at least a subset of the plurality of vesicles comprises a different biosignature from another subset of the plurality of vesicles. The detection system detect at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different subsets of vesicles, wherein each subset of vesicles comprises a different biosignature. For example, a detection system, such as using one or more methods, processes, and compositions disclosed herein, can be used to detect at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different populations of vesicles.

[00660] The detection system can be configured to assess at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 1000, 2500, 5000, 7500, 10,000, 100,000, 150,000, 200,000, 250,000, 300,000, 350,000, 400,000, 450,000, 500,000, 750,000, or 1,000,000 different biomarkers for one or more vesicles. In some embodiments, the one or more biomarkers are selected from any of **Tables 3-5,** or as disclosed herein. The detection system can be configured to assess a specific population of vesicles, such as vesicles from a specific cell-of-origin, or to assess a plurality of specific populations of vesicles, wherein each population of vesicles has a specific biosignature.

[00661] The detection system can be a low density detection system or a high density detection system. For example, a low density detection system can detect up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 different vesicle populations, whereas a high density detection system can detect at least about 15, 20, 25, 50, or 100 different vesicle populations In another embodiment, a low density detection system can detect up to about 100, 200, 300,

PREOMICS EXHIBIT 1002
Page 1126 of 1861

400, or 500 different biomarkers, whereas a high density detection system can detect at least about 750, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9,000, 10,000, 15,000, 20,000, 25,000, 50,000, or 100,000 different biomarkers. In yet another embodiment, a low density detection system can detect up to about 100, 200, 300, 400, or 500 different biosignatures or biomarker combinations, whereas a high density detection system can detect at least about 750, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9,000, 10,000, 15,000, 20,000, 25,000, 50,000, or 100,000 biosignatures or biomarker combinations.

[00662] The detection system can comprise a probe that selectively hybridizes to a vesicle. The detection system can comprise a plurality of probes to detect a vesicle. In some embodiments, a plurality of probes is used to detect the amount of vesicles in a heterogeneous population of vesicles. In yet other embodiments, a plurality of probes is used to detect a homogeneous population of vesicles. A plurality of probes can be used to isolate or detect at least two different subsets of vesicles, wherein each subset of vesicles comprises a different biosignature.

[00663] A detection system, such as using one or more methods, processes, and compositions disclosed herein, can comprise a plurality of probes configured to detect, or isolate, such as using one or more methods, processes, and compositions disclosed herein at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different subsets of vesicles, wherein each subset of vesicles comprises a different biosignature.

[00664] For example, a detection system can comprise a plurality of probes configured to detect at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different populations of vesicles. The detection system can comprise a plurality of probes configured to selectively hybridize to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 1000, 2500, 5000, 7500, 10,000, 100,000, 150,000, 200,000, 250,000, 300,000, 350,000, 400,000, 450,000, 500,000, 750,000, or 1,000,000 different biomarkers for one or more vesicles. In some embodiments, the one or more biomarkers are selected from any of **Tables 3-5,** or as disclosed herein. The plurality of probes can be configured to assess a specific population of vesicles, such as vesicles from a specific cell-of-origin, or to assess a plurality of specific populations of vesicles, wherein each population of vesicles has a specific biosignature.

[00665] The detection system can be a low density detection system or a high density detection system comprising probes to detect vesicles. For example, a low density detection system can comprise probes to detect up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 different vesicle populations, whereas a high density detection system can comprise probes to detect at least about 15, 20, 25, 50, or 100 different vesicle populations. In another embodiment, a low density detection system can comprise probes to detect up to about 100, 200, 300, 400, or 500 different biomarkers, whereas a high density detection system can comprise probes to detect at least about 750, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9,000, 10,000, 15,000, 20,000, 25,000, 50,000, or 100,000 different biomarkers. In yet another embodiment, a low density detection system can comprise probes to detect up to about 100, 200, 300, 400, or 500 different biosignatures or biomarker combinations, whereas a high density detection system can comprise probes to detect at least about 750, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9,000, 10,000, 15,000, 20,000, 25,000, 50,000, or 100,000 biosignatures or biomarker combinations.

[00666] The probes can be specific for detecting a specific vesicle population, for example a vesicle with a particular biosignature, and as described above. A plurality of probes for detecting prostate specific vesicles is also provided. A plurality of probes can comprise probes for detecting one or more of the biomarkers in **Tables**

PREOMICS EXHIBIT 1002
Page 1127 of 1861

3-5. The plurality of probes can also comprise one or more probes for detecting one or more of the biomarkers in **Tables 3-5.**

[00667] A plurality of probes for detecting one or more miRNAs of a vesicle can comprise probes for detecting one or more of the following miRNAs: miR-9, miR-629, miR-141, miR-671-3p, miR-491, miR-1 82, miR-125a-3p, miR-324-5p, miR-148b, and miR-222. In another embodiment, the plurality of probes comprises one or more probes for detecting EpCam, CD9, PCSA, CD63, CD81, PSMA, B7H3, PSCA, ICAM, STEAP, and EGFR. In some embodiments, the plurality of probes comprises one or more probes for detecting EpCam, CD9, PCSA, CD63, CD81, PSMA, and B7H3. In other embodiments, the plurality of probes comprises one or more probes for detecting EpCam, CD9, PCSA, CD63, CD81, PSMA, B7H3, PSCA, ICAM, STEAP, and EGFR. In yet another embodiment, a subset of the plurality of probes are capture agents for one or more of EpCam, CD9, PCSA, CD63, CD81, PSMA, B7H3, PSCA, ICAM, STEAP, and EGFR, and another subset are probes for detecting one or more of CD9, CD63, and CD81 . A plurality of probes can also comprises one or more probes for detecting r miR-92a-2*, miR-147, miR-574-5p, or a combination thereof. A plurality of probes can also comprise one or more probes for detecting miR-548c-5p, miR-362-3p, miR-422a, miR-597, miR-429, miR-200a, miR-200b or a combination thereof. A plurality of probes can also comprise one or more probes for detecting EpCam, CK, and CD45. In some embodiments, the one or more probes may be capture agents. In another embodiment, the probes may be detection agents. In yet another embodiment, the plurality of probes comprises capture and detection agents.

[00668] The probes, such as capture agents, may be attached to a solid substrate, such as an array or bead. Alternatively, the probes, such as detection agents, are not attached. The detection system may be an array based system, a sequencing system, a PCR-based system, or a bead-based system, such as described above. The detection system can also be a microfluidic device as described above.

[00669] The detection system may be part of a kit. Alternatively, the kit may comprise the one or more probe sets or plurality of probes, as described herein. The kit may comprise probes for detecting a vesicle or a plurality of vesicles, such as vesicles in a heterogeneous population. The kit may comprise probes for detecting a homogeneous population of vesicles. For example, the kit may comprise probes for detecting a population of specific cell-of-origin vesicles, or vesicles with the same specific biosignature

Computer Systems

[00670] A vesicle can be assayed for molecular features, for example, by determining an amount, presence or absence of one or more biomarkers. The data generated can be used to produce a biosignature, which can be stored and analyzed by a computer system, such as shown in **FIG. 3.** The assaying or correlating of the biosignature with one or more phenotypes can also be performed by computer systems, such as by using computer executable logic.

[00671] A computer system, such as shown in **FIG. 3,** can be used to transmit data and results following analysis. Accordingly, **FIG. 3** is a block diagram showing a representative example logic device through which results from a vesicle can be analyzed and the analysis reported or generated. **FIG. 3** shows a computer system (or digital device) **800** to receive and store data generated from a vesicle, analyze of the data to generate one or more biosignatures, and produce a report of the one or more biosignatures or phenotype characterization. The computer system can also perform comparisons and analyses of biosignatures generated, and transmit the

PREOMICS EXHIBIT 1002
Page 1128 of 1861

results. Alternatively, the computer system can receive raw data of vesicle analysis, such as through transmission of the data over a network, and perform the analysis.

[00672] The computer system **800** may be understood as a logical apparatus that can read instructions from media **811** and/or network port **805,** which can optionally be connected to server **809** having fixed media **812.** The system shown in **FIG. 3** includes CPU **801,** disk drives **803,** optional input devices such as keyboard **815** and/or mouse **816** and optional monitor **807.** Data communication can be achieved through the indicated communication medium to a server **809** at a local or a remote location. The communication medium can include any means of transmitting and/or receiving data. For example, the communication medium can be a network connection, a wireless connection or an internet connection. Such a connection can provide for communication over the World Wide Web. It is envisioned that data relating to the present invention can be transmitted over such networks or connections for reception and/or review by a party **822.** The receiving party **822** can be but is not limited to an individual, a health care provider or a health care manager. Thus, the information and data on a test result can be produced anywhere in the world and transmitted to a different location. For example, when an assay is conducted in a differing building, city, state, country, continent or offshore, the information and data on a test result may be generated and cast in a transmittable form as described above. The test result in a transmittable form thus can be imported into the U.S. to receiving party **822.** Accordingly, the present invention also encompasses a method for producing a transmittable form of information on the diagnosis of one or more samples from an individual. The method comprises the steps of (1) determining a diagnosis, prognosis, theranosis or the like from the samples according to methods of the invention; and (2) embodying the result of the determining step into a transmittable form. The transmittable form is the product of the production method. In one embodiment, a computer-readable medium includes a medium suitable for transmission of a result of an analysis of a biological sample, such as biosignatures. The medium can include a result regarding a vesicle, such as a biosignature of a subject, wherein such a result is derived using the methods described herein.

EXAMPLES

Example 1: Purification of Vesicles From Prostate Cancer Cell Lines

[00673] Prostate cancer cell lines are cultured for 3-4 days in culture media containing 20% FBS (fetal bovine serum) and 1% P/S/G. The cells are then pre-spun for 10 minutes at 400x g at 4°C. The supernatant is kept and centrifuged for 20 minutes at 2000 x g at 4. The supernatant containing vesicles can be concentrated using a Millipore Centricon Plus-70 (Cat # UFC7 10008 Fisher).

[00674] The Centricon is pre washed with 30mls of PBS at 1000 x g for 3 minutes at room temperature. Next, 15- 70 mis of the pre-spun cell culture supernatant is poured into the Concentrate Cup and is centrifuged in a Swing Bucket Adapter (Fisher Cat # 75-008-144) for 30 minutes at 1000 x g at room temperature.

[00675] The flow through in the Collection Cup is poured off. The volume in the Concentrate Cup is brought back up to 60mls with any additional supernatant. The Concentrate Cup is centrifuged for 30 minutes at 1000 x g at room temperature to concentrate the cell supernatant.

[00676] The Concentrate Cup is washed by adding 70mls of PBS and centrifuged for 30-60 minutes at 1000 x g until approximately 2 mis remains. The vesicles are removed from the filter by inverting the concentrate into the small sample cup and centrifuge for 1 minute at 4°C. The volume is brought up to 25 mis with PBS. The vesicles are now concentrated and are added to a 30% Sucrose Cushion.

PREOMICS EXHIBIT 1002
Page 1129 of 1861

[00677] To make a cushion, 4 mis of Tris/30%Sucrose/D2O solution (30g protease-free sucrose, 2.4g Tris base, 50ml D20, adjust pH to 7.4 with 10N NCL drops, adjust volume to lOOmls with D20, sterilize by passing thru a 0.22-um filter) is loaded to the bottom of a 30ml V bottom thin walled Ultracentrifuge tube. The diluted 25 mis of concentrated vesicles is gently added above the sucrose cushion without disturbing the interface and is centrifuged for 75 minutes at 100,000 x g at 4°C. The ~25mls above the sucrose cushion is carefully removed with a 10ml pipet and the ~3.5mls of vesicles is collected with a fine tip transfer pipet (SAMCO 233) and transferred to a fresh ultracentrifuge tube, where 30 mis PBS is added. The tube is centrifuged for 70 minutes at 100,000 x g at 4°C. The supernatant is poured off carefully. The pellet is resuspended in 200ul PBS and can be stored at 4°C or used for assays. A BCA assay (1:2) can be used to determine protein content and Western blotting or electron micrography can be used to determine vesicle purification.

Example 2: Purification of Vesicles from VCaP and 22Ryl

[00678] Vesicles from Vertebral-Cancer of the Prostate (VCaP) and 22Rvl, a human prostate carcinoma cell line, derived from a human prostatic carcinoma xenograft (CWR22R) were collected by ultracentrifugation by first diluting plasma with an equal volume of PBS (1 ml). The diluted fluid was transferred to a 15 ml falcon tube and centrifuged 30 minutes at 2000 x g 4°C. The supernatant (~2 mis) was transferred to an ultracentrifuge tube 5.0 ml PA thinwall tube (Sorvall # 03 127) and centrifuged at 12,000 x g, 4°C for 45 minutes.

[00679] The supernatant (~2 mis) was transferred to a new 5.0 ml ultracentrifuge tubes and filled to maximum volume with addition of 2.5 mis PBS and centrifuged for 90 minutes at 110,000 x g, 4°C. The supernatant was poured off without disturbing the pellet and the pellet resuspended with 1 ml PBS. The tube was filled to maximum volume with addition of 4.5 ml of PBS and centrifuged at 110,000 x g, 4°C for 70 minutes.

[00680] The supernatant was poured off without disturbing the pellet and an additional 1 ml of PBS was added to wash the pellet. The volume was increased to maximum volume with the addition of 4.5 mis of PBS and centrifuged at 110,000 x g for 70 minutes at 4°C. The supernatant was removed with P-1000 pipette until ~ 100 μΐ of PBS was in the bottom of the tube. The ~ 90 μΐ remaining was removed with P-200 pipette and the pellet collected with the ~10 μΐ of PBS remaining by gently pipetting using a P-20 pipette into the microcentrifuge tube. The residual pellet was washed from the bottom of a dry tube with an additional 5 μΐ of fresh PBS and collected into microcentrifuge tube and suspended in phosphate buffered saline (PBS) to a concentration of 500 μg/πιΐ.

Example 3: Plasma Collection and Vesicle Purification

[00681] Blood is collected via standard veńipuncture in a 7ml K2-EDTA tube. The sample is spun at 400g for 10 minutes in a 4°C centrifuge to separate plasma from blood cells (SORVALL Legend RT+ centrifuge). The supernatant (plasma) is transferred by careful pipetting to 15ml Falcon centrifuge tubes. The plasma is spun at 2,000g for 20 minutes and the supernatant is collected.

[00682] For storage, approximately 1ml of the plasma (supernatant) is aliquoted to a cryovials, placed in dry ice to freeze them and stored in -80°C. Before vesicle purification, if samples were stored at -80°C, samples are thawed in a cold water bath for 5 minutes. The samples are mixed end over end by hand to dissipate insoluble material.

[00683] In a first prespin, the plasma is diluted with an equal volume of PBS (example, approximately 2 ml of plasma is diluted with 2 ml of PBS). The diluted fluid is transferred to a 15 ml Falcon tube and centrifuged for 30 minutes at 2000 x g at 4°C.

PREOMICS EXHIBIT 1002
Page 1130 of 1861

[00684] For a second prespin, the supernatant (approximately 4 mis) is carefully transferred to a 50 ml Falcon tube and centrifuged at 12,000 x g at 4°C for 45 minutes in a Sorval.

[00685] In the isolation step, the supernatant (approximately 2 mis) is carefully transferred to a 5.0 ml ultracentrifuge PA thinwall tube (Sorvall # 03127) using a P1000 pipette and filled to maximum volume with an additional 0.5 mis of PBS. The tube is centrifuged for 90 minutes at 110,000 x g at 4°C.

[00686] In the first wash, the supernatant is poured off without disturbing the pellet. The pellet is resuspended or washed with 1 ml PBS and the tube is filled to maximum volume with an additional 4.5 ml of PBS. The tube is centrifuged at 110,000 x g at 4°C for 70 minutes. A second wash is performed by repeating the same steps.

[00687] The vesicles are collected by removing the supernatant with P-1000 pipette until approximately 100 µï of PBS is in the bottom of the tube. Approximately 90 µï 1of the PBS is removed and discarded with P-200 pipette. The pellet and remaining PBS is collected by gentle pipetting using a P-20 pipette. The residual pellet is washed from the bottom of the dry tube with an additional 5 µï of fresh PBS and collected into a microcentrifuge tube.

Example 4: Analysis of Vesicles Using Antibody-Coupled Microspheres and Directly Conjugated Antibodies

[00688] This example demonstrates the use of particles coupled to an antibody, where the antibody captures the vesicles. See, e.g., **FIG. 2B.** An antibody, the detector antibody, is directly coupled to a label, and is used to detect a biomarker on the captured vesicle.

[00689] First, an antibody-coupled microsphere set is selected (Luminex, Austin, TX). The microsphere set can comprise various antibodies, and thus allows multiplexing. The microspheres are resuspended by vortex and sonication for approximately 20 seconds. A Working Microsphere Mixture is prepared by diluting the coupled microsphere stocks to a final concentration of 100 microspheres of each set/µL in Startblock (Pierce (37538)). 50 µL of Working Microsphere Mixture is used for each well. Either PBS-1% BSA or PBS-BN (PBS, 1% BSA, 0.05% Azide, pH 7.4) may be used as Assay Buffer.

[00690] A 1.2 µπι Millipore filter plate is pre-wet with 100 µï/well of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and aspirated by vacuum manifold. An aliquot of 50 µï of the Working Microsphere Mixture is dispensed into the appropriate wells of the filter plate (Millipore Multiscreen HTS (MSBVN1250)). A 50 µï aliquot of standard or sample is dispensed into to the appropriate wells. The filter plate is covered and incubated for 60 minutes at room temperature on a plate shaker. The plate is covered with a sealer, placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that the speed is set to 550 for the duration of the incubation.

[00691] The supernatant is aspirated by vacuum manifold (less than 5 inches Hg in all aspiration steps). Each well is washed twice with 100 µï of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirated by vacuum manifold. The microspheres are resuspended in 50 µL of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))). The PE conjugated detection antibody is diluted to 4 µg/mL (or appropriate concentration) in PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))). (Note: 50 µL of diluted detection antibody is required for each reaction.) A 50 µï aliquot of the diluted detection antibody is added to each well. The filter plate is covered and incubated for 60 minutes at room temperature on a plate shaker. The filter plate is covered with a sealer, placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that the speed is set to 550 for the duration of the incubation. The supernatant is

PREOMICS EXHIBIT 1002
Page 1131 of 1861

aspirated by vacuum manifold. The wells are washed twice with 100 µï of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and aspirated by vacuum manifold. The microspheres are resuspended in 100 µï of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))). The microspheres are analyzed on a Luminex analyzer according to the system manual.

Example 5: Analysis of Vesicles Using Antibody-Coupled Microspheres and Biotinylated Antibody

[00692] This example demonstrates the use of particles coupled to an antibody, where the antibody captures the vesicles. An antibody, the detector antibody, is biotinylated. A label coupled to streptavidin is used to detect the biomarker.

[00693] First, the appropriate antibody-coupled microsphere set is selected (Luminex, Austin, TX). The microspheres are resuspended by vortex and sonication for approximately 20 seconds. A Working Microsphere Mixture is prepared by diluting the coupled microsphere stocks to a final concentration of 50 microspheres of each set^L in Startblock (Pierce (37538)). (Note: 50 µï of Working Microsphere Mixture is required for each well.) Beads in Start Block should be blocked for 30 minutes and no more than 1 hour.

[00694] A 1.2 µm Millipore filter plate is pre-wet with 100 µï /well of PBS-1% BSA + Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirated by vacuum manifold. A 50 µï aliquot of the Working Microsphere Mixture is dispensed into the appropriate wells of the filter plate (Millipore Multiscreen HTS (MSBVN1250)). A 50 µï aliquot of standard or sample is dispensed to the appropriate wells. The filter plate is covered with a seal and is incubated for 60 minutes at room temperature on a plate shaker. The covered filter plate is placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that, the speed is set to 550 for the duration of the incubation.

[00695] The supernatant is aspirated by a vacuum manifold (less than 5 inches Hg in all aspiration steps). Aspiration can be done with the Pall vacuum manifold. The valve is place in the full off position when the plate is placed on the manifold. To aspirate slowly, the valve is opened to draw the fluid from the wells, which takes approximately 3 seconds for the 100 µï of sample and beads to be fully aspirated from the well. Once the sample drains, the purge button on the manifold is pressed to release residual vacuum pressure from the plate.

[00696] Each well is washed twice with 100 µï of PBS-1% BSA + Azide (PBS-BN)(Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirates by vacuum manifold. The microspheres are resuspended in 50 µï of PBS-1% BSA+ Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032)))

[00697] The biotinylated detection antibody is diluted to 4 µg/mL in PBS-1% BSA + Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))). (Note: 50 µï of diluted detection antibody is required for each reaction.) A 50 µï aliquot of the diluted detection antibody is added to each well.

[00698] The filter plate is covered with a sealer and is incubated for 60 minutes at room temperature on a plate shaker. The plate is placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that, the speed is set to 550 for the duration of the incubation.

[00699] The supernatant is aspirated by vacuum manifold. Aspiration can be done with the Pall vacuum manifold. The valve is place in the full off position when the plate is placed on the manifold. To aspirate slowly, the valve is opened to draw the fluid from the wells, which takes approximately 3 seconds for the 100 ul of sample and beads to be fully aspirated from the well. Once all of the sample is drained, the purge button on the manifold is pressed to release residual vacuum pressure from the plate.

PREOMICS EXHIBIT 1002
Page 1132 of 1861

[00700] Each well is washed twice with 100 µï of PBS-1% BSA + Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirated by vacuum manifold. The microspheres are resuspended in 50 µï of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))).

[00701] The streptavidin-R-phycoerythrin reporter (Molecular Probes 1 mg/ml) is diluted to 4 µg/mL in PBS-1% BSA+ Azide (PBS-BN). 50 µï of diluted streptavidin-R-phycoerythrin was used for each reaction. A 50 µï aliquot of the diluted streptavidin-R-phycoerythrin is added to each well.

[00702] The filter plate is covered with a sealer and is incubated for 60 minutes at room temperature on a plate shaker. The plate is placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that, the speed is set to 550 for the duration of the incubation.

[00703] The supernatant is aspirated by vacuum manifold. Aspiration can be done with the Pall vacuum manifold. The valve is place in the full off position when the plate is placed on the manifold. To aspirate slowly, the valve is opened to draw the fluid from the wells, which takes approximately 3 seconds for the 100 ul of sample and beads to be fully aspirated from the well. Once all of the sample is drained, the purge button on the manifold is pressed to release residual vacuum pressure from the plate.

[00704] Each well is washed twice with 100 µï of PBS-1% BSA + Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirated by vacuum manifold. The microspheres are resuspended in 100 µï of PBS-1% BSA+ Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and analyzed on the Luminex analyzer according to the system manual.

Example 6: Vesicle Concentration from Plasma

[00705] Supplies and Equipment: Pall life sciences Acrodisc, 25mm syringe filter w/1.2 um, Versapor membrane (sterile) Part number: 4190; Pierce concentrators 7 ml/150 K MWCO (molecular weight cut off), Part number: 89922; BD syringe filter, 10 ml, Part number: 305482; Sorvall Legend RT Plus Series Benchtop Centrifuge w 15 ml swinging bucket rotor; PBS, pH 7.4, Sigma cat#P3813-10PAK prepared in Sterile Molecular grade water; Co-polymer 1.7ml microfuge tubes, USA Scientific, cat#1415-2500. Water used for reagents is Sterile Filtered Molecular grade water (Sigma, cat#W4502). Handling of patient plasma is done in a biosafety hood.

Procedure:

1. Filter procedure for plasma samples

1.1. Remove plasma samples from -80°C (-65°C to -85°C) freezer
1.2. Thaw samples in room temperature water (10-15 minutes).
1.3. Prepare syringe and filter by removing the number necessary from their casing.
1.4. Pull plunger to draw 4mL of sterile molecular grade water into the syringe. Attach a 1.2µηι filter to the syringe tip and pass contents through the filter onto the 7 ml/150 K MWCO Pierce column.
1.5. Cap the columns and place in the swing bucket centrifuge at spin at lOOOxg in Sorvall Legend RT plus centrifuge for 4 minutes at 20°C (16°C-24°C).
1.6. While spinning, disassemble the filter from syringe. Then remove plunger from syringe.
1.7. Discard flow through from the tube and gently tap column on paper towels to remove any residual water.
1.8. Measure and record starting volumes for all plasma samples. Samples with a volume less than 900µ1may not be processed.
1.9. Place open syringe and filter on open Pierce column. Fill open end of syringe with 5.2mL of IX PBS and pipette mix plasma into PBS three to four times.

PREOMICS EXHIBIT 1002
Page 1133 of 1861

1.10. Replace the plunger of the syringe and slowly depress the plunger until the contents of the syringe have passed through the filter onto the Pierce column. Contents should pass through the filter drop wise.

2. Microvesicle concentration centrifugation protocol

2.1. Spin 7 ml/150 K MWCO Pierce columns at 2000xg at 20°C (16°C-24°C) for 60 minutes or until volume is reduced to 250-300 µL. If needed, spin for additional 15 minutes increments to reach required volume.

2.2. At the conclusion of the spin, pipette mix on the column 15x (avoid creating bubbles) and withdraw volume (300µL or less) and transfer to a new 1.7mL co-polymer tube.

2.3. The final volume of the plasma concentrate is dependent on the initial volume of plasma. Plasma is concentrated to 300ul if the original plasma volume is lml. If the original volume of plasma is less than lml, then the volume of concentrate should be consistent with that ratio. For example, if the original volume is 900ul, then the volume of concentrate is 270ul. The equation to follow is: x=(y/1000)*300, where x is the final volume of concentrate and y is the initial volume of plasma.

2.4. Record the sample volume and add IX PBS to the sample to make the final sample volume.

2.5. Store concentrated microvesicle sample at 4°C (2°C to 8°C).

Calculations:

1. Final volume of concentrated plasma sample
x=(y/1000)*300, where x is the final volume of concentrate and y is the initial volume of plasma.

Example 7: Capture of Vesicles Using Magnetic Beads

[00706] Vesicles isolated as described in **Example 2** are used. Approximately 40 µl of the vesicles are incubated with approximately 5 µg (~50 µl) of EpCam antibody coated Dynal beads (Invitrogen, Carlsbad, CA) and 50 µl of Starting Block. The vesicles and beads are incubated with shaking for 2 hours at 45°C in a shaking incubator. The tube containing the Dynal beads is placed on the magnetic separator for 1 minute and the supernatant removed. The beads are washed twice and the supernatant removed each time. Wash beads twice, discarding the supernatant each time.

Example 8: Detection of mRNA Transcripts in Vesicles

[00707] RNA from the bead-bound vesicles of **Example 7** was isolated using the Qiagen miRneasy™ kit, (Cat. No. 217061), according to the manufacturer's instructions.

[00708] The vesicles are homogenized in QIAzol™ Lysis Reagent (Qiagen Cat. No. 79306). After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase, while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase. The upper, aqueous phase is extracted, and ethanol is added to provide appropriate binding conditions for all RNA molecules from 18 nucleotides (nt) upwards. The sample is then applied to the RNeasy™ Mini spin column, where the total RNA binds to the membrane and phenol and other contaminants are efficiently washed away. High quality RNA is then eluted in RNase-free water.

[00709] RNA from the VCAP bead captured vesicles was measured with the Taqman TMPRSS:ERG fusion transcript assay *(Kirsten D. Mertz et al. Neoplasia. 2007 March; 9(3): 200-206.)*. RNA from the 22Rvl bead captured vesicles was measured with the Taqman SPINK1 transcript assay *(Scott A. Tomlins et al. Cancer Cell 2008 June 13(6):519-528)*. The GAPDH transcript (control transcript) was also measured for both sets of vesicle RNA.

PREOMICS EXHIBIT 1002
Page 1134 of 1861

[00710] Higher CT values indicate lower transcript expression. One change in cycle threshold (CT) is equivalent to a 2 fold change, 3 CT difference to a 4 fold change, and so forth, which can be calculated with the following: $2^{\wedge CT1-CT2.}$ This experiment shows a difference in CT of the expression of the fusion transcript TMPRSS:ERG and the equivalent captured with the IgG2 negative control bead **(FIG. 5).** The same comparison of the SPINK1 transcript in 22RV1 vesicles showed a CT difference of 6.14 for a fold change of 70.5. Results with GAPDH were similar (not shown).

Example 9: Obtaining Serum Samples from Subjects

[00711] Blood is collected from subjects (both healthy subjects and subjects with cancer) in EDTA tubes, citrate tubes or in a 10 ml Vacutainer SST plus Blood Collection Tube (BD367985 or BD366643, BD Biosciences). Blood is processed for plasma isolation within 2 h of collection.

[00712] Samples are allowed to sit at room temperature for a minimum of 30 min and a max of 2 h. Separation of the clot is accomplished by centrifugation at 1,000-1,300 x *g* at 4°C for 15-20 min. The serum is removed and dispensed in aliquots of 500 µï into 500 to 750 µï cryotubes. Specimens are stored at -80°C.

[00713] At a given sitting, the amount of blood drawn can range from ~20 to ~90 ml. Blood from several EDTA tubes is pooled and transferred to RNase/DNase-free 50-ml conical tubes (Greiner), and centrifuged at 1,200 x *g* at room temperature in a Hettich Rotanta 460R benchtop centrifuge for 10 min. Plasma is transferred to a fresh tube, leaving behind a fixed height of 0.5 cm plasma supernatant above the pellet to avoid disturbing the pellet. Plasma is aliquoted, with inversion to mix between each aliquot, and stored at -80°C.

Example 10: RNA Isolation From Human Plasma and Serum Samples

[00714] Four hundred µï of human plasma or serum is thawed on ice and lysed with an equal volume of 2X Denaturing Solution (Ambion). RNA is isolated using the *mirVana* PARIS kit following the manufacturer's protocol for liquid samples (Ambion), modified such that samples are extracted twice with an equal volume of acid-phenol chloroform (as supplied by the Ambion kit). RNA is eluted with 105 µï of Ambion elution solution according to the manufacturer's protocol. The average volume of eluate recovered from each column is about 80 µï.

[00715] A scaled-up version of the *mirVana* PARIS (Ambion) protocol is also used: 10 ml of plasma is thawed on ice, two 5-ml aliquots are transferred to 50-ml tubes, diluted with an equal volume of *mirVana* PARIS 2X Denaturing Solution, mixed thoroughly by vortexing for 30 s and incubated on ice for 5 min. An equal volume (10 ml) of acid/phenol/chloroform (Ambion) is then added to each aliquot. The resulting solutions are vortexed for 1 min and spun for 5 min at 8,000 rpm, 20°C in a JA17 rotor. The acid/phenol/chloroform extraction is repeated three times. The resulting aqueous volume is mixed thoroughly with 1.25 volumes of 100% molecular-grade ethanol and passed through a *mirVana* PARIS column in sequential 700-µl aliquots. The column is washed following the manufacturer's protocol, and RNA is eluted in 105 µï of elution buffer (95°C). A total of 1.5 µï of the eluate is quantified by Nanodrop.

Example 11: Measurement of miRNA Levels in RNA from Plasma and Serum using qRT-PCR

[00716] A fixed volume of 1.67 µï of RNA solution from about ~80 µï -eluate from RNA isolation of a given sample is used as input into the reverse transcription (RT) reaction. For samples in which RNA is isolated from a 400- µï plasma or serum sample, for example, 1.67 µï of RNA solution represents the RNA corresponding to (1.67/80) X 400 = 8.3 µï plasma or serum. For generation of standard curves of chemically synthesized RNA

PREOMICS EXHIBIT 1002
Page 1135 of 1861

oligonucleotides corresponding to known miRNAs, varying dilutions of each oligonucleotide are made in water such that the final input into the RT reaction has a volume of 1.67 μΐ. Input RNA is reverse transcribed using the TaqMan miRNA Reverse Transcription Kit and miRNA-specific stem-loop primers (Applied BioSystems) in a small-scale RT reaction comprised of 1.387 μΐ of H20, 0.5 μΐ of 10X Reverse-Transcription Buffer, 0.063 μΐ of RNase-Inhibitor (20 units / μΐ), 0.05 μΐ of 100 mM dNTPs with dTTP, 0.33 μΐ of Multiscribe Reverse-Transcriptase, and 1.67 μΐ of input RNA; components other than the input RNA can be prepared as a larger volume master mix, using a Tetrad2 Peltier Thermal Cycler (BioRad) at 16°C for 30 min, 42°C for 30 min and 85°C for 5 min. Real-time PCR is carried out on an Applied BioSystems 7900HT thermocycler at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Data is analyzed with SDS Relative Quantification Software version 2.2.2 (Applied BioSystems.), with the automatic Ct setting for assigning baseline and threshold for Ct determination.

[00717] The protocol can also be modified to include a preamplification step, such as for detecting miRNA. A 1.25-μ1 aliquot of undiluted RT product is combined with 3.75 μΐ of Preamplification PCR reagents [comprised, per reaction, of 2.5 μΐ of TaqMan PreAmp Master Mix (2X) and 1.25 μΐ of 0.2X TaqMan miRNA Assay (diluted in TE)] to generate a 5.0-μ1 preamplification PCR, which is carried out on a Tetrad2 Peltier Thermal Cycler (BioRad) by heating to 95°C for 10 min, followed by 14 cycles of 95°C for 15 s and 60°C for 4 min. The preamplification PCR product is diluted (by adding 20 μΐ of H20 to the 5-μ1 preamplification reaction product), following which 2.25 μΐ of the diluted material is introduced into the real-time PCR and carried forward as described.

Example 12: Extracting microRNA from Vesicles

[00718] MicroRNA is extracted from vesicles isolated from patient samples as described herein. See, e.g., **Example 6.** Methods for isolation and concentration of vesicles are presented herein. The methods in this Example can also be used to isolate microRNA from patient samples without first isolating vesicles.

[00719] Protocol Using Trizol

[00720] This protocol uses the QIAzol Lysis Reagent and RNeasy Midi Kit from Qiagen Inc., Valencia CA to extract microRNA from concentrated vesicles. The steps of the method comprise:

1. Add 2 μΐ of RNase A to 50 μΐ of vesicle concentrate, incubate at 37°C for 20 min.
2. Add 700 μΐ of QIAzol Lysis Reagent, vortex 1 minute. Spike samples with 25 &no1/μL of *C. elegans* microRNA (1 μL) after the addition of QIAzol, making a 75 &no1/μE spike in for each total sample (3 aliquots combined).
3. Incubate at 55°C for 5 min.
4. Add 140 μΐ chloroform and shake vigorously for 15 sec.
5. Cool on ice for 2-3 min.
6. Centrifuge @ 12,000 x g at 4°C for 15 min.
7. Transfer aqueous phase (300 μL) to a new tube and add 1.5 volumes of 100% EtOH (i.e., 450 μL).
8. Pipet up to 4 ml of sample into an RNeasy Midi spin column in a 15 ml collection tube (combining lysis from 3 50 μΐ of concentrate)
9. Spin at 2700 x g for 5 min at room temperature.
10. Discard flowthrough from the spin.
11. Add 1 ml of Buffer RWT to column and centrifuge at 2700 x g for 5 min at room temperature. Do not use Buffer RW1 supplied in the Midi kit. Buffer RW1 can wash away miRNA. Buffer RWT is supplied in the Mini kit from Qiagen Inc.
12. Discard flowthrough.
13. Add 1 ml of Buffer RPE onto the column and centrifuge at 2700 x g for 2 min at room temperature.
14. Repeat steps 12 and 13.
16. Place column into a new 15 ml collection tube and add 150 μΐ Elution Buffer. Incubate at room temperature for 3 min.

PREOMICS EXHIBIT 1002
Page 1136 of 1861

17. Centrifuge at 2700 x g for 3 min at room temperature.
18. Vortex the sample and transfer to 1.7 mL tube. Store the extracted sample at -80°C.

[00721] Modified Trizol Protocol

1. Add Epicentre RNase A to final concentration of 229 µg/ml (Epicentre®, an Illumina® company, Madison, WI). (For example, to 150 ul of concentrate, add 450 µl PBS and 28.8 µl Epicentre Rnase A [5µg/µl].) Vortex briefly. Incubate for 20 min at 37°C. Aliquot "babies" in increments of 100 µl using reverse pipetting.
2. Set temperature on centrifuge to 4°C.
3. Add 750 µl of Trizol LS to each 100 µl sample and immediately vortex.
5. Incubate on benchtop at room temperature (RT) for 5 mins.
6. Vortex all samples for 30 min. at 1400 rpm at RT in the MixMate. While vortexing, add BCP phase separation agent to the plate.
7. Briefly centrifuge tubes. Transfer the sample to the collection microtube rack.
8. Add 150 µl BCP to the samples in the plate. Cap the plate and shake vigorously for 15 sec.
9. Incubate at RT for 3 min.
10. Centrifuge at 6,000 x g at 4°C for 15 min. Reset centrifuge temperature to 24°C (RT).
11. Add 500 µl 100% EtOH to the appropriate wells of a new S-block. Transfer 200 µl aqueous phase to new S-block, mix the aqueous/EtOH by pipetting 10X.
12. Briefly centrifuge.
13. Place an RNeasy 96 (Qiagen, Inc., Valencia, CA) plate on top of a new S-block. Pipette the aqueous/EtOH sample mixture into the wells of the RNeasy 96 plate. Seal the RNeasy 96 plate with AirPore tape.
14. Spin at 6000 rpm (-5600 x g) for 4 min at RT. Avoid temps below 24°C.
15. Empty the S-block by discarding the flowthrough and remove the AirPore tape.
14. Add 700 µl of Buffer RWT to the plate, seal with AirPore tape, and centrifuge at 6,000 rpm for 4 min at RT. Empty the S-block and remove the AirPore tape.
15. Add 500 µl of Buffer RPE to the plate, seal with AirPore tape, and centrifuge at 6,000 rpm for 4 min at RT. Empty the S-block and remove the AirPore tape.
16. Add another 500 µl of Buffer RPE to the plate, seal with AirPore tape, and centrifuge at 6,000 rpm for 10 min at RT. Empty the S-block and remove the AirPore tape.
17. Place the Rneasy 96 plate on top of a clean elution microtube rack. Pipet 30 µl of RNase-free water onto the columns of the Rneasy 96 plate. Seal with AirPore tape.
18. Allow water to sit on column for 5 min.
19. Centrifuge column for 4 min at 6,000 rpm to elute RNA. Cap the microtubes with elution microtube caps. Pool babies together.
20. Store @ -80°C.

[00722] Protocol using MagMax

[00723] This protocol uses the MagMAX™ RNA Isolation Kit from Applied Biosystems/Ambion, Austin, TX to extract microRNA from concentrated vesicles. The steps of the method comprise:

1. Add 700 ml of QIAzol Lysis Reagent and vortex 1 minute.
2. Incubate on benchtop at room temperature for 5 min.
3. Add 140 µl chloroform and shake vigorously for 15 sec.
4. Incubate on benchtop for 2-3 min.
5. Centrifuge at 12,000 x g at 4°C for 15 min.
6. Transfer aqueous phase to a deep well plate and add 1.25 volumes of 100% Isopropanol.
7. Shake MagMAX™ binding beads well. Pipet 10 µl of RNA binding beads into each well.
8. Gather two elution plates and two additional deep well plates.
9. Label one elution plate "Elution" and the other "Tip Comb."
10. Label one deep well as "1st Wash 2" and the other as "2nd Wash 2."
11. Fill both Wash 2 deep well plates with 150 µl of Wash 2, being sure to add ethanol to wash beforehand. Fill in the same number of wells as there are samples.
12. Select the appropriate collection program on the MagMax Particle Processor.
13. Press start and load each appropriate plate.
14. Transfer samples to microcentrifuge tubes.
15. Vortex and store at -80°C. Residual beads will be seen in sample.

PREOMICS EXHIBIT 1002
Page 1137 of 1861

Example 13: MicroRNA Arrays

[00724] MicroRNA levels in a sample can be analyzed using an array format, including both high density and low density arrays. Array analysis can be used to discover differentially expressed in a desired setting, e.g., by analyzing the expression of a plurality of miRs in two samples and performing a statistical analysis to determine which ones are differentially expressed between the samples and can therefore be used in a biosignature. The arrays can also be used to identify a presence or level of one or more microRNAs in a single sample in order to characterize a phenotype by identifying a biosignature in the sample. This Example describes commercially available systems that are used to carry out the methods of the invention.

[00725] TaqMan Low Density Array

[00726] TaqMan Low Density Array (TLDA) miRNA cards are used to compare expression of miRNA in various sample groups as desired. The miRNA are collected and analyzed using the TaqMan® MicroRNA Assays and Arrays systems from Applied Biosystems, Foster City, CA. Applied Biosystems TaqMan® Human MicroRNA Arrays are used according to the Megaplex™ Pools Quick Reference Card protocol supplied by the manufacturer.

[00727] Exiqon mIRCURY LNA microRNA

[00728] The Exiqon miRCURY LNA™ Universal RT microRNA PCR Human Panels I and II (Exiqon, Inc, Woburn, MA) are used to compare expression of miRNA in various sample groups as desired. The Exiqon 384 well panels include 750 miRs. Samples are normalized to control primers towards synthetic RNA spike-in from Universal cDNA synthesis kit (UniSp6 CP). Results were normalized to inter-plate calibrator probes.

[00729] With either system, quality control standards are implemented. Normalized values for each probe across three data sets for each indication are averaged. Probes with an average CV% higher than 20% are not used for analysis. Results are subjected to a paired t-test to find differentially expressed miRs between two sample groups. P-values are corrected with a Benjamini and Hochberg false-discovery rate test. Results are analyzed using using GeneSpring software (Agilent Technologies, Inc., Santa Clara, CA).

Example 14: MicroRNA Profiles in Vesicles

[00730] Vesicles were collected by ultracentrifugation from 22Rvl, LNCaP, Vcap and normal plasma (pooled from 16 donors) as described in **Examples 1-3.** RNA was extracted using the Exiqon miR isolation kit (Cat. Nos. 3001 10, 3001 11). Equals amounts of vesicles (30µg) were used as determined by BCA assay.

[00731] Equal volumes (5 µĩ) were put into a reverse-transcription reaction for microRNA. The reverse-transcriptase reactions were diluted in 81 µĩ of nuclease-free water and then 9 µĩ of this solution was added to each individual miR assay. MiR-629 was found to only be expressed in PCa (prostate cancer) vesicles and was virtually undetectable in normal plasma vesicles. MiR-9 was found to be highly overexpressed (-704 fold increase over normal as measured by copy number) in all PCa cell lines, and has very low expression in normal plasma vesicles.

Example 15: MicroRNA Profiles of Magnetic EpCam-Captured Vesicles

[00732] The bead-bound vesicles of **Example 7** were placed in QIAzol™ Lysis Reagent (Qiagen Cat. #79306). An aliquot of 125 fmol of c. *elegans* miR-39 was added. The RNA was isolated using the Qiagen miRneasy™ kit, (Cat. # 217061), according to the manufacturer's instructions, and eluted in 30 ul RNAse free water.

[00733] 10 µĩ of the purified RNA was placed into a pre-amplification reaction for miR-9, miR-141 and miR-629 using a Veriti 96-well thermocycler. A 1:5 dilution of the pre-amplification solution was used to set up a

PREOMICS EXHIBIT 1002
Page 1138 of 1861

qRT-PCR reaction for miR9 (ABI 4373285), miR-141 (ABI 4373 137) and miR-629 (ABI 4380969) as well as *c. elegans* miR-39 (ABI 4373455). The results were normalized to the *c. elegans* results for each sample.

Example 16: MicroRNA Profiles of CD9-Captured Vesicles

[00734] CD9 coated Dynal beads (Invitrogen, Carlsbad, CA) were used instead of EpCam coated beads as in **Example 15.** Vesicles from prostate cancer patients, LNCaP, or normal purified vesicles were incubated with the CD9 coated beads and the RNA isolated as described in **Example 15.** The expression of miR-21 and miR-141 was detected by qRT-PCR and the results depicted in **FIG. 6.**

Example 17: Isolation of Vesicles Using a Filtration Module

[00735] Six mL of PBS is added to 1 mL of plasma. Optionally, the sample can be treated with a blocking agent such as StabilGuard®, which may improve downstream processing. The sample is then put through a 1.2 micron (μm) Pall syringe filter directly into a 100 kDa MWCO (Millipore, Billerica, MA), 7 ml column with a 150 kDa MWCO (Pierce®, Rockford, IL), 15 ml column with a 100 kDa MWCO (Millipore, Billerica, MA), or 20 ml column with a 150 kDa MWCO (Pierce®, Rockford, IL).

[00736] The tube is centrifuged for between 60 to 90 minutes until the volume is about 250 μΐ. The retentate is collected and PBC added to bring the sample up to 300 μΐ. Fifty μΐ of the sample is then used for further vesicle analysis, such as further described in the examples below.

Example 18: Multiplex Analysis of Vesicles Isolated with Filters

[00737] The vesicle samples obtained using methods as described in **Example 17** are used in multiplexing assays as described herein. See, e.g., **Examples 23-24** below. The capture antibodies are CD9, CD63, CD81, PSMA, PCSA, B7H3, and EpCam. The detection antibodies are for biomarkers CD9, CD81, and CD63 or B7H3 and EpCam.

Example 19: Flow Cytometry Analysis of Vesicles

[00738] Purified plasma vesicles are assayed using the MoFlo XDP (Beckman Coulter, Fort Collins, CO, USA) and the median fluorescent intensity analyzed using the Summit 4.3 Software (Beckman Coulter). Vesicles are labeled directly with antibodies, or beads or microspheres (e.g., magnetic, polystyrene, including BD FACS 7-color setup, catalog no. 335775) can be incorporated. Vesicles can be detected with binding agents against the following vesicle antigens: CD9 (Mouse anti-human CD9, MAB1 880, R&D Systems, Minneapolis, MN, USA), PSM (Mouse anti-human PSM, sc-7365 1, Santa Cruz, Santa Cruz, CA, USA), PCSA (Mouse anti-human Prostate Cell Surface Antigen, MAB4089, Millipore, MA, USA), CD63 (Mouse anti-human CD63, 556019, BD Biosciences, San Jose, CA, USA), CD81 (Mouse anti-human CD81, 555675, BD Biosciences, San Jose, CA, USA) B7-H3 (Goat anti-human B7-H3, AF1027, R&D Systems, Minneapolis, MN, USA), EpCAM (Mouse anti-human EpCAM, MAB9601, R&D Systems, Minneapolis, MN, USA). Vesicles can be detected with fluorescently labeled antibodies against the desired vesicle antigens. For example, FITC, phycoerythrin (PE) and Cy7 are commonly used to label the antibodies.

[00739] To capture the antibodies with multiplex microspheres, the microspheres can be obtained from Luminex (Austin, TX, USA) and conjugated to the desired antibodies using micros using Sulfo-NHS and EDC obtained from Pierce Thermo (Cat. No. 245 10 and 22981, respectively, Rockford, 111, USA).

[00740] Purified vesicles (lOug/ml) are incubated with 5,000 microspheres for one hour at room temperature with shaking. The samples are washed in FACS buffer (0.5% FBS/PBS) for 10 minutes at 1700 rpms. The

PREOMICS EXHIBIT 1002
Page 1139 of 1861

detection antibodies are incubated at the manufacturer's recommended concentrations for one hour at room temperature with shaking. Following another wash with FACS buffer for 10 minutes at 1700 rpms, the samples are resuspended in lOOul FACS buffer and run on the FACS machine.

[00741] Further when using microspheres to detect vesicles, the labeled vesicles can be sorted according to their detection antibody content into different tubes. For example, using FITC or PE labeled microspheres, a first tube contains the population of microspheres with no detectors, the second tube contains the population with PE detectors, the third tube contains the population with FITC detectors, and the fourth tube contains the population with both PE and FITC detectors. The sorted vesicle populations can be further analyzed, e.g., by examining payload such as mRNA, microRNA or protein content.

[00742] **FIG. 7A** shows separation and identification of vesicles using the MoFlo XDP. In this set of experiments, there were about 3000 trigger events with just buffer (i.e. particulates about the size of a large vesicle). There were about 46,000 trigger events with unstained vesicles (43,000 vesicles of sufficient size to scatter the laser). There were 500,000 trigger events with stained vesicles. Vesicles were detected using detection agents for tetraspanins CD9, CD63, and CD81 all labeled with FITC. The smaller vesicles can be detected when they are stained with detection agents.

[00743] **FIG. 7B** shows FACS analysis of VCaP cells (left panels) and VCaP exosomes (right panels) for CD9, B7H3, PSMA and PCSA. The analysis demonstrated that both VCaP cells and VCaP-derived exosomes shared similar surface protein markers. Cytofluorometric analysis using flow cytometry revealed that both the VCaP cells and the VCaP-derived vesicles contained CD9, CD63, CD81, PCSA, PSMA and B7-H3 antigens that were accessible to PE-labeled antibodies. Antigens at a lower concentration on the cell surface can be found at a higher concentration on the microvesicle surface (e.g. PCSA).

[00744] The microRNA content in flow sorted miRs can differ depending on the marker used to detect the vesicles. VCaP-derived vesicles were sorted using labeled antibodies to B7H3 or PSMA. miR expression patterns in the captured vesicles were determined using Exiqon cards as described herein. **FIG. 7C** shows that different patterns of expression were obtained in B7H3+ or PSMA+ vesicle populations as compared to overall vesicle population.

[00745] Physical isolation by sorting of specific populations of vesicles facilitates additional studies such as microRNA analysis on the partially or wholly purified vesicle populations.

Example 20: Antibody Detection of Vesicles

[00746] Vesicles in a patient sample are assessed using antibody-coated beads to detect the vesicles in the sample using techniques as described herein. The following general protocol is used:

a. Blood is drawn from a patient at a point of care (e.g., clinic, doctor's office, hospital).

b. The plasma fraction of the blood is used for further analysis.

c. To remove large particles and isolate a vesicle containing fraction, the plasma sample is filtered, e.g., with a 0.8 or 1.2 micron (μm) syringe filter, and then passed through a size exclusion column, e.g., with a 150 kDa molecular weight cut off. A general schematic is shown in **FIG. 8A.** Filtration may be preferable to ultracentrifugation, as illustrated in **FIG. 8B.** Without being bound by theory, high-speed centrifugation may remove protein targets weakly anchored in the membrane as opposed to the tetraspanins which are more solidly

PREOMICS EXHIBIT 1002
Page 1140 of 1861

anchored in the membrane, and may reduce the cell specific targets in the vesicle, which would then not be detected in subsequent analysis of the biosignature of the vesicle.

d. The vesicle fraction is incubated with beads conjugated with a "capture" antibody to a marker of interest. The captured vesicles are then tagged with labeled "detection" antibodies, e.g., phycoerythrin or FITC conjugated antibodies. The beads can be labeled as well.

e. Captured and tagged vesicles in the sample are detected. Fluorescently labeled beads and detection antibodies can be detected as shown in **FIG. 8C.** Use of the labeled beads and labeled detection antibodies allows assessment of beads with vesicles bound thereto by the capture antibody. Note that the figure is simplified for purposes of illustration. For example, different detectors can be used for each laser.

f. Data is analyzed. A threshold can be set for the median fluorescent intensity (MFI) of a particular capture antibody. A reading for that capture antibody above the threshold can indicate a certain phenotype. As an illustrative example, an MFI above the threshold for a capture antibody directed to a cancer marker can indicate the presense of cancer in the patient sample.

[00747] In **FIG. 8C,** the beads **816** flow through a capillary **811.** Use of dual lasers **812** at different wavelengths allows separate detection at detector **813** of both the capture antibody **818** from the fluorescent signal derived from the bead, as well as the median fluorescent intensity (MFI) resulting from the labeled detection antibodies **819.** Use of labeled beads conjugated to different capture antibodies of interest, each bead labeled with a different fluor, allows for mulitiplex analysis of different vesicle **817** populations in a single assay as shown. Laser 1 **815** allows detection of bead type (i.e., the capture antibody) and Laser 2 **814** allows measurement of detector antibodies, which can include general vesicle markers such as tetraspanins including CD9, CD63 and CD81 . Use of different populations of beads and lasers allows simultaneous multiplex analysis of many different populations of vesicles in a single assay.

[00748] **FIG. 8D** represents an example of detecting prostate-cancer derived vesicles bound to a substrate using the general protocol in this Example. The microvesicles are captured with capture agents specific to PCSA, PSMA or B7H3 tethered to the substrate (i.e., beads). The so-captured vesicles are labeled with fluorescently labeled detection agents specific to tetraspanins CD9, CD63 and CD81 .

[00749] The MFI values obtained using the microsphere assay correlate with the levels of the target proteins as determined by alternate methods. Levels of VCap derived vesicles were compared between the microsphere assay, FACS, and BCA protein assay. Analysis of CD9-labeled vesicles demonstrated tight correlation between MFI and number of vesicles as determined by Flow analysis, as shown in **FIG. 8E.** Analysis using PSMA, PCSA and B7H3 as vesicle markers showed that total protein concentration from VCaP vesicles measured using the BCA protein assay also correlated tightly to the MFI value determined on the microvesicle assay, as shown in **FIG. 8F.**

[00750] The microsphere assay can be used to detect markers in a multiplex format without hinderence in assay performance. For example, we found no competition effect observed by the multiplexing of 6 different capture antibodies (PSMA, PCSA, B7-H3, CD9, CD63, CD81). The MFIs recorded for the multiplexed method were identical to the MFIs recorded for each individual marker when run in a single-plex assay format. Comparison of the distribution of MFI values obtained using the cMV-based assay that used multiplexed antibodies with one

PREOMICS EXHIBIT 1002
Page 1141 of 1861

that included a single antibody against the biomarker CD81 are shown in **FIG. 8G.** Frequency is expressed as the normalized number of beads. Singleplex vs multiplex B7H3, CD63, CD9, and EpCam capture antibody comparisons also showed no interference in a multiplex format at two different non-saturating VCaP vesicle concentrations, as shown in **FIG. 8H.**

Example 21: Detection of Prostate Cancer

[00751] High quality training set samples were obtained from commercial suppliers. The samples comprised plasma from 42 normal prostate, 42 PCa and 15 BPH patients. The PCa samples included 4 stage III and the remainder state II. The samples were blinded until all laboratory work was completed.

[00752] The vesicles from the samples were obtained by filtration to eliminate particles greater than 1.5 microns, followed by column concentration and purification using hollow fiber membrane tubes. The samples were analyzed using a multiplexed bead-based assay system as described above.

[00753] Antibodies to the following proteins were analyzed:

a. General Vesicle (MV) markers: CD9, CD8 1, and CD63
b. Prostate MV markers: PCSA
c. Cancer-Associated MV markers: EpCam and B7H3

[00754] Samples were required to pass a quality test as follows: if multiplexed median fluorescence intensity (MFI) PSCA + MFI B7H3 + MFI EpCam < 200 then sample fails due to lack of signal above background. In the training set, six samples (three normals and three prostate cancers) did not achieve an adequate quality score and were excluded. An upper limit on the MFI was also established as follows: if MFI of EpCam is > 6300 then test is over the upper limit score and samples are deemed not cancer (i.e., "negative" for purposes of the test).

[00755] The samples were classified according to the result of MFI scores for the six antibodies to the training set proteins, wherein the following conditions must be met for the sample to be classified as PCa positive:

a. Average MFI of General MV markers > 1500
b. PCSA MFI > 300
c. B7H3 MFI > 550
d. EpCam MFI between 550 and 6300

[00756] Using the 84 normal and PCa training data samples, the test was found to be 98% sensitive and 95% specific for PCa vs normal samples. See **FIG. 9A.** The increased MFI of the PCa samples compared to normals is shown in **FIG. 9B.** Compared to PSA and PCA3 testing, the PCa Test presented in this Example can result in saving -220 men without PCa in every 1000 normal men screened from having an unnecessary biopsy.

Example 22: Microsphere Vesicle Prostate Cancer Assay Protocol

[00757] In this example, the vesicle PCa test is a microsphere based immunoassay for the detection of a set of protein biomarkers present on the vesicles from plasma of patients with prostate cancer. The test employs specific antibodies to the following protein biomarkers: CD9, CD59, CD63, CD81, PSMA, PCSA, B7H3 and EpCAM. After capture of the vesicles by antibody coated microspheres, phycoerythrin-labeled antibodies are used for the detection of vesicle specific biomarkers. Depending on the level of binding of these antibodies to the vesicles from a patient's plasma a determination of the presence or absence of prostate cancer is made.

[00758] Vesicles are isolated as described above.

[00759] *Microspheres*

PREOMICS EXHIBIT 1002
Page 1142 of 1861

[00760] Specific antibodies are conjugated to microspheres (Luminex) after which the microspheres are combined to make a Microsphere Master Mix consisting of L100-C105-01 ; L100-C1 15-01 ; L100-C1 19-01 ; L100-C120-01 ; L100-C122-01 ; L100-C124-01 ; L100-C135-01 ; and L100-C175-01 . xMAP® Classification Calibration Microspheres L100-CAL1 (Luminex) are used as instrument calibration reagents for the Luminex LX200 instrument. xMAP® Reporter Calibration Microspheres L100-CAL2 (Luminex) are used as instrument reporter calibration reagents for the Luminex LX200 instrument. xMAP® Classification Control Microspheres L100-CON1 (Luminex) are used as instrument control reagents for the Luminex LX200 instrument. xMAP Reporter Control Microspheres L100-CON2 (Luminex) and are used as reporter control reagents for the Luminex LX200 instrument.

[00761] *Capture Antibodies*

[00762] The following antibodies are used to coat Luminex microspheres for use in capturing certain populations of vesicles by binding to their respective protein targets on the vesicles in this Example: a. Mouse anti-human CD9 monoclonal antibody is an IgG2b used to coat microsphere L100-C105 to make *EPCLMACD9-C105; b. Mouse anti-human PSMA monoclonal antibody is an IgGl used to coat microsphere L100-C1 15 to make EPCLMAPSMA-Cl 15; c. Mouse anti-human PCSA monoclonal antibody is an IgGl used to coat microsphere L100-C1 19 to make EPCLMAPCSA-C1 19; d. Mouse anti-human CD63monoclonal antibody is an IgGl used to coat microsphere L100-C120 to make EPCLMACD63-C120; e. Mouse anti-human CD81 monoclonal antibody is an IgGl used to coat microsphere L100-C124 to make EPCLMACD81-C124; f. Goat anti-human B7-H3 polyclonal antibody is an IgG purified antibody used to coat microsphere L100-C125 to make EPCLGAB7-H3-C125; and g. Mouse anti-human EpCAM monoclonal antibody is an IgG2b purified antibody used to coat microsphere L100-C175 to make EPCLMAEpCAM-C175.

[00763] *Detection Antibodies*

[00764] The following phycoerythrin (PE) labeled antibodies are used as detection probes in this assay: a. EPCLMACD81PE: Mouse anti-human CD81 PE labeled antibody is an IgGl antibody used to detect CD81 on captured vesicles; b. EPCLMACD9PE: Mouse anti-human CD9 PE labeled antibody is an IgGl antibody used to detect CD9 on captured vesicles; c. EPCLMACD63PE: Mouse anti-human CD63 PE labeled antibody is an IgGl antibody used to detect CD63 on captured vesicles; d. EPCLMAEpCAMPE: Mouse anti-human EpCAM PE labeled antibody is an IgGl antibody used to detect EpCAM on captured vesicles; e. EPCLMAPSMAPE: Mouse anti-human PSMA PE labeled antibody is an IgGl antibody used to detect PSMA on captured vesicles; f. EPCLMACD59PE: Mouse anti-human CD59 PE labeled antibody is an IgGl antibody used to detect CD59 on captured vesicles; and g. EPCLMAB7-H3PE: Mouse anti-human B7-H3 PE labeled antibody is an IgGl antibody used to detect B7-H3 on captured vesicles.

[00765] *Reagent Preparation*

[00766] Antibody Purification: The following antibodies in **Table 12** are received from vendors and purified and adjusted to the desired working concentrations according to the following protocol.

Table 12: Antibodies for PCa Assay

Antibody	Use
EPCLMACD9	Coating of microspheres for vesicle capture
EPCLMACD63	Coating of microspheres for vesicle capture
EPCLMACD81	Coating of microspheres for vesicle capture
EPCLMAPSMA	Coating of microspheres for vesicle capture
EPCLGAB7-H3	Coating of microspheres for vesicle capture

PREOMICS EXHIBIT 1002
Page 1143 of 1861

EPCLMAEpCAM	Coating of microspheres for vesicle capture
EPCLMAPCSA	Coating of microspheres for vesicle capture
EPCLMACD81PE	PE coated antibody for vesicle biomarker detection
EPCLMACD9PE	PE coated antibody for vesicle biomarker detection
EPCLMACD63PE	PE coated antibody for vesicle biomarker detection
EPCLMAEpCAMPE	PE coated antibody for vesicle biomarker detection
EPCLMAPSMAPE	PE coated antibody for vesicle biomarker detection
EPCLMACD59PE	PE coated antibody for vesicle biomarker detection
EPCLMAB7-H3PE	PE coated antibody for vesicle biomarker detection

[00767] Antibody Purification Protocol: Antibodies are purified using Protein G resin from Pierce (Protein G spin kit, prod # 89979). Micro-chromatography columns made from filtered P-200 tips are used for purification.

[00768] One hundred µĩ of Protein G resin is loaded with IOOµI buffer from the Pierce kit to each micro column. After waiting a few minutes to allow the resin to settle down, air pressure is applied with a P-200 Pipettman to drain buffer when needed, ensuring the column is not let to dry. The column is equilibrated with 0.6ml of Binding Buffer (pH 7.4, lOOmM Phosphate Buffer, 150mM NaCl; (Pierce, Prod # 89979). An antibody is applied to the column (<lmg of antibody is loaded on the column). The column is washed with 1.5ml of Binding Buffer. Five tubes (1.5 ml micro centrifuge tubes) are prepared and 10 µĩ of neutralization solution (Pierce, Prod # 89979) is applied to each tube. The antibody is eluted with the elution buffer from the kit to each of the five tubes, lOOul for each tube (for a total of 500 µĩ). The relative absorbance of each fraction is measured at 280nm using Nanodrop (Thermo scientific, Nanodrop 1000 spectrophotometer). The fractions with highest OD reading are selected for downstream usage. The samples are dialyzed against 0.25 liters PBS buffer using Pierce Slide-A-Lyzer Dialysis Cassette (Pierce, prod 66333, 3KDa cut off). The buffer is exchanged every 2 hours for minimum three exchanges at 4°C with continuous stirring. The dialyzed samples are then transferred to 1.5ml microcentifuge tubes, and can be labeled and stored at 4°C (short term) or -20°C (long term).

[00769] Microsphere Working Mix Assembly : A microsphere working mix MWM101 includes the first four rows of antibody, microsphere and coated microsphere of **Table 13.**

Table 13: Antibody-Microsphere Combinations

Antibody	Microsphere	Coated Microsphere
EPCLMACD9	L100-C105	EPCLMACD9-C105
EPCLMACD63	L100-C120	EPCLMACD63-C120
EPCLMACD81	L100-C124	EPCLMACD81-C124
EPCLMAPSMA	L100-C115	EPCLMAPSMA-C115
EPCLGAB7-H3	L100-C125	EPCLGAB7-H3-C125
bEPCLMAEpCAM	L100-C175	EPCLMAEpCAM-C175
EPCLMAPCSA	L100-C119	EPCLMAPCSA-C119

[00770] Microspheres are coated with their respective antibodies as listed above according to the following protocol.

[00771] Protocol for Two-Step Carbodiimide Coupling of Protein to Carboxylated Microspheres: The microspheres should be protected from prolonged exposure to light throughout this procedure. The stock uncoupled microspheres are resuspended according to the instructions described in the Product Information Sheet provided with the microspheres (xMAP technologies, MicroPlex TM Microspheres). Five x 106 of the stock microspheres are transferred to a USA Scientific 1.5ml microcentrifuge tube. The stock microspheres are pelleted by microcentrifugation at ≥ 8000 x g for 1-2 minutes at room temperature. The supernatant is removed

PREOMICS EXHIBIT 1002
Page 1144 of 1861

and the pelleted microspheres are resuspended in 100 µï of dH20 by vortex and sonication for approximately 20 seconds. The microspheres are pelleted by microcentrifugation at ≥ 8000 x g for 1-2 minutes at room temperature. The supernatant is removed and the washed microspheres are resuspended in 80 µï of 100 mM Monobasic Sodium Phosphate, pH 6.2 by vortex and sonication (Branson 15 10, Branson UL Trasonics Corp.) for approximately 20 seconds. Ten µï of 50 mg/ml Sulfo-NHS (Thermo Scientific, Cat#24500) (diluted in dH20) is added to the microspheres and is mixed gently by vortex. Ten µï of 50 mg/ml EDC (Thermo Scientific, Cat# 25952-53-8) (diluted in dH20) is added to the microspheres and gently mixed by vortexing. The microspheres are incubated for 20 minutes at room temperature with gentle mixing by vortex at 10 minute intervals. The activated microspheres are pelleted by microcentrifugation at ≥ 8000 x g for 1-2 minutes at room temperature. The supernatant is removed and the microspheres are resuspended in 250 µï of 50 mM MES, pH 5.0 (MES, Sigma, Cat# M2933) by vortex and sonication for approximately 20 seconds. (Only PBS-1% BSA+ Azide (PBS-BNX (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) should be used as assay buffer as well as wash buffer.). The microspheres are then pelleted by microcentrifugation at ≥ 8000 x g for 1-2 minutes at room temperature.

[00772] The supernatant is removed and the microspheres are resuspended in 250 µï of 50 mM MES, pH 5.0 (MES, Sigma, Cat# M2933) by vortex and sonication for approximately 20 seconds. (Only PBS-1% BSA+ Azide (PBS-BN) ((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) should be used as assay buffer as well as wash buffer.). The microspheres are then pelleted by microcentrifugation at ≥ 8000 x g for 1-2 minutes at room temperature, thus completing two washes with 50 mM MES, pH 5.0.

[00773] The supernatant is removed and the activated and washed microspheres are resuspended in 100 µï of 50 mM MES, pH 5.0 by vortex and sonication for approximately 20 seconds. Protien in the amount of 125, 25, 5 or 1 µg is added to the resuspended microspheres. (Note: Titration in the 1 to 125 µg range can be performed to determine the optimal amount of protein per specific coupling reaction.). The total volume is brought up to 500 µï with 50 mM MES, pH 5.0. The coupling reaction is mixed by vortex and is incubated for 2 hours with mixing (by rotating on Labquake rotator, Bamstead) at room temperature. The coupled microspheres are pelleted by microcentrifugation at ≥ 8000 x g for 1-2 minutes at room temperature. The supernatant is removed and the pelleted microspheres are resuspended in 500 µL of PBS-TBN by vortex and sonication for approximately 20 seconds. (Concentrations can be optimized for specific reagents, assay conditions, level of multiplexing, etc. in use.).

[00774] The microspheres are incubated for 30 minutes with mixing (by rotating on Labquake rotator, Bamstead) at room temperature. The coupled microspheres are pelleted by microcentrifugation at ≥ 8000 x g for 1-2 minutes at room temperature. The supernatant is removed and the microspheres are resuspended in 1 ml of PBS-TBN by vortex and sonication for approximately 20 seconds. (Each time there is the addition of samples, detector antibody or SA-PE the plate is covered with a sealer and light blocker (such as aluminum foil), placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that the speed should be set to 550 for the duration of the incubation.).

[00775] The microspheres are pelleted by microcentrifugation at ≥ 8000 x g for 1-2 minutes. The supernatant is removed and the microspheres are resuspended in 1 ml of PBS-TBN by vortex and sonication for approximately 20 seconds. The microspheres are pelleted by microcentrifugation at ≥ 8000 x g for 1-2 minutes (resulting in a total of two washes with 1 ml PBS-TBN).

PREOMICS EXHIBIT 1002
Page 1145 of 1861

[00776] *Protocol for microsphere assay:* The preparation for multiple phycoerythrin detector antibodies is used as described in **Example 4.** One hundred µĩ is analyzed on the Luminex analyzer (Luminex 200, xMAP technologies) according to the system manual (High PMT setting).

[00777] *Decision Tree:* A decision tree as in **FIG. 10** is used to assess the results from the microsphere assay to determine if a subject has cancer. Threshold limits on the MFI is established and samples classified according to the result of MFI scores for the antibodies, to determine whether a sample has sufficient signal to perform analysis (e.g., is a valid sample for analysis or an invalid sample for further analysis, in which case a second patient sample may be obtained) and whether the sample is PCa positive. **FIG. 10** shows a decision tree using the MFI obtained with PCSA, PSMA, B7-H3, CD9, CD81 and CD63. A sample is classified as indeterminate if the MFI is within the standard deviation of the predetermined threshold (TH). In this case, a second patient sample can be obtained. For validation, the sample must have sufficient signal when capturing vesicles with the individual tetraspanins and labeling with all tetraspanins. A sample that passes validation is called positive if either of the prostate-specific markers (PSMA or PCSA) is considered positive, and the cancer marker (B7-H3) is also considered positive.

[00778] *Results:* See **Example 23.**

Example 23: Microsphere Vesicle PCa Assay Performance

[00779] In this example, the vesicle PCa test is a microsphere based immunoassay for the detection of a set of protein biomarkers present on the vesicles from plasma of patients with prostate cancer. The test is performed similarly to that of **Example 22** with modifications indicated below.

[00780] The test uses a multiplexed immunoassay designed to detect circulating microvesicles. The test uses PCSA, PSMA and B7H3 to capture the microvesicles present in patient samples such as plasma and uses CD9, CD81, and CD63 to detect the captured microvesicles. The output of this assay is the median fluorescent intensity (MFI) that results from the antibody capture and fluorescently labeled antibody detection of microvesicles that contain both the individual capture protein and the detector proteins on the microvesicle. A sample is "POSITIVE" by this test if the MFI levels of PSMA or PCSA, and B7H3 protein-containing microvesicles are above the empirically determined threshold. A method for determining the threshold is presented in Example 33 of International Patent Application Serial No. PCT/US201 1/03 1479, entitled "Circulating Biomarkers for Disease" and filed April 6, 201 1, which application is incorporated by reference in its entirety herein. A sample is determined to be "NEGATIVE" if any one of these two microvesicle capture categories exhibit an MFI level that is below the empirically determined threshold. Alternatively, a result of "INDETERMINATE" will be reported if the sample MFI fails to clearly produce a positive or negative result due to MFI values not meeting certain thresholds or the replicate data showed too much statistical variation. A "NON-EVALUABLE" interpretation for this test indicates that this patient sample contained inadequate microvesicle quality for analysis. See Example 33 of International Patent Application Serial No. PCT/US201 1/03 1479 for a method to determine the empirically derived threshold values.

[00781] The test employs specific antibodies to the following protein biomarkers: CD9, CD59, CD63, CD81, PSMA, PCSA, and B7H3 as in **Example 22.** Decision rules are set to determine if a sample is called positive, negative or indeterminate, as outlined in **Table 14.** See also **Example 22.** For a sample to be called positive the replicates must exceed all four of the MFI cutoffs determined for the tetraspanin markers (CD9, CD63, CD81), prostate markers (PSMA or PCSA), and B7H3. Samples are called indeterminate if both of the three replicates

PREOMICS EXHIBIT 1002
Page 1146 of 1861

from PSMA and PCSA or any of the three replicates from B7H3 antibodies span the cutoff MFI value. Samples are called negative if there is at least one of the tetraspanin markers (CD9, CD63, and CD81), prostate markers (PSMA or PCSA), B7H3 that fall below the MFI cutoffs.

Table 14: MFI Parameter for Each Capture Antibody

Tetraspanin Markers (CD9, CD63, CD81)	Prostate Markers (PSMA, PCSA)	B7H3	Result Determination
Average of all replicates from the three tetraspanins have a MFI >500	All replicates from either of the two prostate markers have a MFI >350 for PCSA and >90 for PSMA	All replicates from B7H3 have a MFI >300	If all 3 are true, then the sample is called Positive
	Both replicate sets from either prostate marker have values both above and below a MFI =350 for PCSA and =90 for PSMA	Any replicates from B7H3 have values both above and below a MFI =300	If either are true, then the sample is called indeterminate
All replicates from the three tetraspanins have a MFI <500	All replicates from either of the two prostate markers have a MFI <350 for PCSA and <90 for PSMA	All replicates from B7H3 have a MFI <300	If any of the 3 are true, then the sample is called Negative, given the sample doesn't qualify as indeterminate

[00782] The vesicle PCa test was compared to elevated PSA on a cohort of 296 patients with or without PCa as confirmed by biopsy. An ROC curve of the results is shown in **FIG. 11.** As shown, the area under the curve (AUC) for the vesicle PCa test was 0.94 whereas the AUC for elevated PSA on the same samples was only 0.68. The PCa samples were likely found due to a high PSA value. Thus this population is skewed in favor of PSA, accounting for the higher AUC than is observed in a true clinical setting.

[00783] The vesicle PCa test was further performed on a cohort of 933 patient plasma samples. Results are summarized in **Table 15:**

Table 15: Performance of vesicle PCa test on 933 patient cohort

True Positive	409
True Negative	307
False Positive	50
False Negative	72
Non-evaluable	63
Indeterminate	32
Total	933
Sensitivity	85%
Specificity	86%
Accuracy	85%
Non-evaluable Rate	8%
Indeterminate Rate	5%

PREOMICS EXHIBIT 1002
Page 1147 of 1861

[00784] As shown in **Table 15,** the vesicle PCa test achieved an 85% sensitivity level at a 86% specificity level, for an accuracy of 85%. In contrast, PSA at a sensitivity of 85% had a specificity of about 55%, and PSA at a specificity of 86% had a sensitivity of about 5%. **FIG. 11.** About 12% of the 933 samples were non-evaluable or indeterminate. Samples from the patients could be recollected and re-evaluated. The vesicle PCa test had an AUC of 0.92 for the 933 samples.

Example 24: Vesicle Protein Array to Detect Prostate Cancer

[00785] In this example, the vesicle PCa test is performed using a protein array, more specifically an antibody array, for the detection of a set of protein biomarkers present on the vesicles from plasma of patients with prostate cancer. The array comprises capture antibodies specific to the following protein biomarkers: CD9, CD59, CD63, CD81 . Vesicles are isolated as described above, e.g., in **Example 20.** After filtration and isolation of the vesicles from plasma of men at risk for PCa, such as those over the age of 50, the plasma samples are incubated with an array harboring the various capture antibodies. Depending on the level of binding of fluorescently labeled detection antibodies to PSMA, PCSA, B7H3 and EpCAM that bind to the vesicles from a patient's plasma that hybridize to the array, a determination of the presence or absence of prostate cancer is made.

[00786] In a second array format, the vesicles are isolated from plasma and hybridized to an array containing CD9, CD59, CD63, CD81, PSMA, PCSA, B7H3 and EpCam. The captured vesicles are tagged with non-specific vesicle antibodies labeled with Cy3 and/or Cy5. The fluorescence is detected. Depending on the pattern of binding, a determination of the presence or absence of prostate cancer is made.

Example 25: Distinguishing BPH and PCa using miRs

[00787] RNA from the plasma derived vesicles of nine normal male individuals and nine individuals with stage 3 prostate cancers were analyzed on the Exiqon mIRCURY LNA microRNA PCR system panel. The Exiqon 384 well panels measure 750 miRs. Samples were normalized to control primers towards synthetic RNA spike-in from Universal cDNA synthesis kit (UniSp6 CP). Normalized values for each probe across three data sets for each indication (BPH or PCa) were averaged. Probes with an average CV% higher than 20% were not used for analysis.

[00788] Analysis of the results revealed several microRNAs that were 2 fold or more over-expressed in BPH samples compared to Stage 3 prostate cancer samples. These miRs include: hsa-miR-329, hsa-miR-30a, hsa-miR-335, hsa-miR-152, hsa-miR-15 1-5p, hsa-miR-200a and hsa-miR-145, as shown in **Table 16:**

Table 16: miRs overexpressed in BPH vs PCa

Overexpressed in BPH v PCa	Fold Change
hsa-miR-329	12.32
hsa-miR-30a	6.16
hsa-miR-335	6.00
hsa-miR-152	4.73
hsa-miR-151-5p	3.16
hsa-miR-200a	3.16
hsa-miR-145	2.35

Example 26: miR-145 in controls and PCa samples

[00789] **FIG. 12** illustrates a comparison of miR-145 in control and prostate cancer samples. RNA was collected as in **Example 12.** The controls include Caucasians >75 years old and African Americans >65 years

PREOMICS EXHIBIT 1002
Page 1148 of 1861

old with PSA < 4 ng/ml and a benign digital rectal exam (DRE). As seen in the figure, miR-145 was under expressed in PCa samples. miR-145 is useful for identifying those with early/indolent PCa vs those with benign prostate changes (e.g., BPH).

Example 27: miRs to enhance vesicle diagnostic assay performance

[00790] As described herein, vesicles are concentrated in plasma patient samples and assessed to provide a diagnostic, prognostic or theranostic readout. Vesicle analysis of patient samples includes the detection of vesicle surface biomarkers, e.g., surface antigens, and/or vesicle payload, e.g., mRNAs and microRNAs, as described herein. The payload within the vesicles can be assessed to enhance assay performance. For example, **FIG. 13A** illustrates a scheme for using miR analysis within vesicles to convert false negatives into true positives, thereby improving sensitivity. In this scheme, samples called negative by the vesicle surface antigen analysis are further confirmed as true negatives or true positives by assessing payload with the vesicles. Similarly, **FIG. 13B** illustrates a scheme for using miR analysis within vesicles to convert false positives into true negatives, thereby improving specificity. In this scheme, samples called positive by the vesicle surface antigen analysis are further confirmed as true negatives or true positives by assessing payload with the vesicles.

[00791] A diagnostic test for prostate cancer includes isolating vesicles from a blood sample from a patient to detect vesicles indicative of the presence or absence of prostate cancer. See, e.g., **Examples 20-23.** The blood can be serum or plasma. The vesicles are isolated by capture with "capture antibodies" that recognize specific vesicle surface antigens. The surface antigens for the prostate cancer diagnostic assay include the tetraspanins CD9, CD63 and CD81, which are generally present on vesicles in the blood and therefore act as general vesicle biomarkers, the prostate specific biomarkers PSMA and PCSA, and the cancer specific biomarker B7H3. The capture antibodies are tethered to fluorescently labeled beads, wherein the beads are differentially labeled for each capture antibody. Captured vesicles are further highlighted using fluorescently labeled "detection antibodies" to the tetraspanins CD9, CD63 and CD81. Fluorescence from the beads and the detection antibodies is used to determine an amount of vesicles in the plasma sample expressing the surface antigens for the prostate cancer diagnostic assay. The fluorescence levels in a sample are compared to a reference level that can distinguish samples having prostate cancer. In this Example, microRNA analysis is used to enhance the performance of the vesicle-based prostate cancer diagnostic assay.

[00792] **FIG. 13C** shows the results of detection of miR-107 in samples assessed by the vesicle-based prostate cancer diagnostic assay. **FIG. 13D** shows the results of detection of miR-141 in samples assessed by the vesicle-based prostate cancer diagnostic assay. In the figure, normalized levels of the indicated miRs are shown on the Y axis for true positives (TP) called by the vesicle diagnostic assay, true negatives (TN) called by the vesicle diagnostic assay, false positives (FP) called by the vesicle diagnostic assay, and false negatives (FN) called by the vesicle diagnostic assay. As shown in **FIG. 13C,** the use of miR-107 enhances the sensitivity of the vesicle assay by distinguishing false negatives from true negative ($p=0.0008$). **FIG. 13E** shows verification of increased miR-107 in plasma cMVs of prostate cancer patients compared to patients without prostate cancer using a different sample cohort. Similarly, **FIG. 13D** also shows that the use of miR-141 enhances the sensitivity of the vesicle assay by distinguishing false negatives from true negative ($p=0.0001$). Results of adding miR-141 are shown in **Table 17.** miR-574-3p performs similarly.

Table 17: Addition of miR-141 to vesicle-based test for PCa

	Without miR-141	With miR-141

PREOMICS EXHIBIT 1002
Page 1149 of 1861

Sensitivity	85%	98%
Specificity	86%	86%

[00793] In this Example, vesicles are detected via surface antigens that are indicative of prostate cancer, and the performance of the signature is further bolstered by examining miRs within the vesicles, i.e., sensitivity is increased without negatively affecting specificity. This general methodology can be extended for any setting in which vesicles are profiled for surface antigens or other informative characteristic, then one or more additional biomarker is used to enhance characterization. Here, the one or more additional biomarkers are miRs. They could also comprise mRNA, soluble protein, lipids, carbohydrates and any other vesicle-associated biological entities that are useful for characterizing the phenotype of interest.

Example 28: Vesicle Isolation and Detection Methods

[00794] A number of technologies known to those of skill in the art can be used for isolation and detection of vesicles to carry out the methods of the invention in addition to those described above. The following is an illustrative description of several such methods.

[00795] **Glass Microbeads.** Available as VeraCode / BeadXpress from Illumina, Inc. San Diego, CA, USA. The steps are as follows:

1. Prepare the beads by direct conjugation of antibodies to available carboxyl groups.
2. Block non specific binding sites on the surface of the beads.
3. Add the beads to the vesicle concentrate sample.
4. Wash the samples so that unbound vesicles are removed.
5. Apply fluorescently labeled antibodies as detection antibodies which will bind specifically to the vesicles.
6. Wash the plate, so that the unbound detection antibodies are removed.
7. Measure the fluorescence of the plate wells to determine the presence the vesicles.

[00796] **Enzyme Linked Immunosorbent Assay (ELISA).** Methods of performing ELISA are well known to those of skill in the art. The steps are generally as follows:

1. Prepare a surface to which a known quantity of capture antibody is bound.
2. Block non specific binding sites on the surface.
3. Apply the vesicle sample to the plate.
4. Wash the plate, so that unbound vesicles are removed.
5. Apply enzyme linked primary antibodies as detection antibodies which also bind specifically to the vesicles.
6. Wash the plate, so that the unbound antibody-enzyme conjugates are removed.
7. Apply a chemical which is converted by the enzyme into a color, fluorescent or electrochemical signal.
8. Measure the absorbency, fluorescence or electrochemical signal (e.g., current) of the plate wells to determine the presence and quantity of vesicles.

[00797] **Electrochemiluminescence detection arrays.** Available from Meso Scale Discovery, Gaithersburg, MD, USA:

1. Prepare plate coating buffer by combining 5 mL buffer of choice (e.g. PBS, TBS, HEPES) and 75 µL of 1% Triton X-100 (0.015% final).
2. Dilute capture antibody to be coated.
3. Prepare 5 µL of diluted a capture ntibody per well using plate coating buffer (with Triton).

PREOMICS EXHIBIT 1002
Page 1150 of 1861

4. Apply 5 µL of diluted capture antibody directly to the center of the working electrode surface being careful not to breach the dielectric. The droplet should spread over time to the edge of the dielectric barrier but not cross it.
5. Allow plates to sit uncovered and undisturbed overnight.

[00798] The vesicle containing sample and a solution containing the labeled detection antibody are added to the plate wells. The detection antibody is an anti-target antibody labeled with an electrochemiluminescent compound, MSD SULFO-TAG label. Vesicles present in the sample bind the capture antibody immobilized on the electrode and the labeled detection antibody binds the target on the vesicle, completing the sandwich. MSD read buffer is added to provide the necessary environment for electrochemilummescence detection. The plate is inserted into a reader wherein a voltage is applied to the plate electrodes, which causes the label bound to the electrode surface to emit light. The reader detects the intensity of the emitted light to provide a quantitative measure of the amount of vesicles in the sample.

[00799] **Nanoparticles.** Multiple sets of gold nanoparticles are prepared with a separate antibody bound to each. The concentrated microvesicles are incubated with a single bead type for 4 hours at 37°C on a glass slide. If sufficient quantities of the target are present, there is a colorimetric shift from red to purple. The assay is performed separately for each target. Gold nanoparticles are available from Nanosphere, Inc. of Northbrook, Illinois, USA.

[00800] **Nanosight.** A diameter of one or more vesicles can be determined using optical particle detection. See U.S. Patent 7,75 1,053, entitled "Optical Detection and Analysis of Particles" and issued July 6, 2010; and U.S. Patent 7,399,600, entitled "Optical Detection and Analysis of Particles" and issued July 15, 2010. The particles can also be labeled and counted so that an amount of distinct vesicles or vesicle populations can be assessed in a sample.

Example 29: KRAS sequencing in CRC cell lines and patient samples

[00801] KRAS RNA was isolated from vesicles derived from CRC cell lines and sequenced. RNA was converted to cDNA prior to sequencing. Sequencing was performed on the cell lines listed in **Table 18:**

Table 18: CRC cell lines and KRAS sequence

Cell Line	DNA or Vesicle cDNA	KRAS Genotype Exon 2	KRAS Genotype Exon 3
Colo 205	Vesicle cDNA	Wild type (WT)	WT
Colo 205	DNA	WT	WT
HCT 116	Vesicle cDNA	c.13G>GA	WT
HCT 116	DNA	c.13G>GA	WT
HT29	Vesicle cDNA	WT	WT
Lovo	Vesicle cDNA	c.13G>GA	WT
Lovo	DNA	c.13G>GA	WT
RKO	Vesicle cDNA	WT	WT
SW 620	Vesicle cDNA	c.12G>T	WT

[00802] **Table 18** and **FIG. 14** show that the mutations detected in the genomic DNA from the cell lines was also detected in RNA contained within vesicles derived from the cell lines. **FIG. 14** shows the sequence in HCT 116 cells of cDNA derived from vesicle mRNA in **(FIG. 14A)** and genomic DNA **(FIG. 14B).**

[00803] Twelve CRC patient samples were sequenced for KRAS. As shown in **Table 19,** all were wild type (WT). All patient samples received a DNase treatment during RNA Extraction. RNA was extracted from isolated vesicles. All 12 patients amplified for GAPDH demonstrating RNA was present in their vesicles.

PREOMICS EXHIBIT 1002
Page 1151 of 1861

Table 19: CRC patient samples and KRAS sequence

Sample	Sample Type	Stage	KRAS Genotype Exon 2	KRAS Genotype Exon 3
61473a6	Colon Ca	1	WT	WT
62454a4	Colon Ca	1	WT	WT
110681a4	Colon Ca	1	WT	Failed sequencing
28836a7	Colon Ca	1	WT	Failed sequencing
62025a2	Colon Ca	2a	WT	WT
62015a4	Colon Ca	2a	WT	WT
110638a3	Colon Ca	2a	WT	WT
110775a3	Colon Ca	2a	WT	WT
35512a5	Colon Ca	3	WT	WT
73231a1	Colon Ca	2a	WT	WT
85823a3	Colon Ca	3b	WT	WT
23440a7	Colon Ca	3c	WT	WT
145151A2/3	Normal		WT	WT
139231A3	Normal		WT	Failed sequencing
145155A4	Normal		WT	Failed sequencing
145154A4	Normal		WT	Failed sequencing

[00804] In a patient sample wherein the patient was found positive for the KRAS 13G>A mutation, the KRAS mutation from the tumor of CRC patient samples could also be identified in plasma-derived vesicles from the same patient. **FIG. 14** shows the sequence in this patient of cDNA derived from vesicle mRNA in plasma **(FIG. 14C)** and also genomic DNA derived from a fresh frozen paraffin embedded (FFPE) tumor sample **(FIG. 14D).**

Example 30: Immunoprecipitation of Protein - Nucleic Acid Complexes

[00805] This Example examined the levels of miRNAs in plasma contained in complexes with Ago2, Apolipoprotein AI, and GW182. Specifically, miRNA levels were assessed after co-immunoprecipitation with antibodies to Ago2, Apolipoprotein AI, and GW182.

[00806] To carry out the immunoprecipitation, human plasma was incubated with antibodies bound to protein G beads against Ago2, Apolipoprotein AI, GW182, and an IgG control. To prepare the beads, 10 µg of anti-AG02 (ab57113, lot GR291717-1, Abeam, Cambridge, MA), anti-ApoAI (PA1-22558, Thermo Scientific, Waltham, MA), anti-GW182 (A302-330A, Bethyl Labs, Montgomery, TX) or anti-IgG (sc-2025, Santa Cruz, Santa Cruz, CA) were conjugated to Magnabind protein G beads (Cat. # 21349, Thermo Scientific) or Dynabead Protein G (Cat. # 100.04D, Invitrogen, Carlsbad, CA). 200 µl of beads were placed in a 1.5 ml eppendorf tube and placed on a magnetic separator (Cat. # S1509S, New England Biolabs, Ipswich, MA) for one minute. The storage buffer was removed and discarded. The beads were washed once with 200 ml of phosphate buffered saline (PBS). The antibodies were allowed to bind the beads in 200 µl PBS for 30 minutes at room temperature (RT) and then for an additional 90 minutes at 4°C. The antibody-bound beads were placed on the magnetic separator for one minute. Unbound antibody was removed and discarded. The beads were washed three times with ice cold PBS.

[00807] The antibody conjugated beads were resuspended in 200 µl of PBS and mixed with 200 µl of human plasma from normal subjects (i.e., without cancer). The mixture was allowed to roll overnight on a Thermo Scientific Labquake Shaker/Rotisserie at 4°C. Following the overnight incubation, the beads were placed on the magnetic separator for 1 minute or until the solution turned clear. The beads were washed three times with 200

PREOMICS EXHIBIT 1002
Page 1152 of 1861

μΐ cold PBS and once with 200 μΐ of an NP-40 wash buffer (1% NP-40, 50 mM Tris-HCl, pH 7.4, 150 mM NaCl and 2 mM EDTA). Following the NP-40 buffer wash, the samples were rinsed one additional time with 200 μΐ of cold PBS. The beads were placed on the magnetic separator for one minute. The beads were the brought back to the original starting volume in 200 μΐ of PBS. Three quarters of the sample was used for RNA isolation as described previously (Arroyo et al., 201 1). The remaining was stored at -20°C for Western analysis.

[00808] The isolated RNA was screened for miR-16 and miR-92a using ABI Taqman detection kits ABI_391 and ABI_43 1, respectively (Applied Biosystems, Carlsbad, CA). RNA was quantified against synthetic standards. The supernatant was collected and analyzed for selected miRNAs (miR-16 and miR-92a). The levels of miR-16 and miR-92a detected are shown in **FIG. 15.** As shown in the **FIG. 15A** and **FIG. 15B,** respectively, miR-16 and miR-92a co-immunoprecipitated with Ago2 and GW1 82 using Magnabeads at much higher levels than the IgG control (compare bars denoted as "Beads"). Co-immunoprecipitation with Dynabeads was unsuccessful for technical reasons which were not explored further.

[00809] Potential source(s) of miRNA from human plasma include vesicles and/or circulating Ago2-bound ribonucleoprotein complexes (RNP). miRs can be simultaneously isolated from complexes with AGOl-4 and vesicles using capture of GW1 82. This Example shows that miR-16 and miR-92a co-immunoprecipitate with AG02 and GW1 82 in human plasma.

Example 31: Flow Analysis and Sorting of cells, vesicles and protein-nucleic acid complexes

[00810] This Example provides protocols for flow analysis and sorting of cells, circulating microvesicles (cMVs), and protein-nucleic acid complexes. Any appropriate antibody can be used that recognizes the markers of interest. The protocols can be applied to different sample sources, such as analysis of cells, vesicles and complexes from cell culture or from various bodily fluids.

[00811] 1) Flow sorting microRNA complexes

[00812] Circulating microRNA derived from specific tissues can be isolated using tissue specific biomarkers to isolate the microvesicles and other microRNA complexes. This Example shows that microRNA in a PCSA/Ago2 double positive sub-population in human plasma can distinguish prostate cancer from non-cancer.

[00813] Plasma samples from three subjects with prostate cancer and three male subjects without prostate cancer were treated to concentrate vesicles as in **Example 17.** The concentrated vesicles were stained using optimized concentrations of antibodies against PCSA, a prostate specific biomarker, and Ago2 (ab571 13, lot GR291 17-1, Abeam, Cambridge, MA). The antibodies used were anti-PCSA labeled with PE and anti-Ago2 labeled with FITC. Positive gates were set using matching isotype control antibodies to define positive and negative regions. Sorted populations were selected based on regions as shown in **FIG. 16.** The Beckman Coulter MoFlo-XDP cell sorter and flow cytometer was used to isolated positive events using the high-purity sorting mode (i.e., "Purify 1/ Drop") to ensure that sorted events were pure to >90%. The MoFlo-XDP is capable of sorting two populations at rates of up to 50,000 events per second. To ensure purity and efficiency of the particle sort, the rate was between 200-300 events per second on average. Positive events were sorted into three 2 ml tubes and reserved for subsequent miR analysis.

[00814] Once sorted, the microRNA content from each prostate specific subpopulation was evaluated. When a comparison of total concentrated plasma-derived microvesicles was made, little differential expression of miR-22 was observed between prostate cancer (PrC) and non-cancer samples (i.e., normals) **(FIG. 17A).** Similar results were observed with mean copy number levels of miR-22 from total RNA isolated from each PCSA/Ago2

PREOMICS EXHIBIT 1002
Page 1153 of 1861

double population **(FIG. 17B).** Without taking microRNA levels into account, the number of PCSA/Ago2 double positive events from each plasma sample did not significantly distinguish cancer from non-cancer **(FIG. 17C).** However, a clear separation was observed between prostate cancer and non-cancer when the number of observed copies of miR-22 from each sort was divided by the specific number of events from each sort **(FIG. 17D).** In this latter case, higher levels of miR-22 per PCSA/Ago2 double positive complexes were observed in all PCa plasma samples as compared to normal.

[00815] The protocol can be extended to detect and/or sort cMVs by detecting vesicles with anti-tetraspanin antibodies to first recognize cMVs. For example, the sample can first be sorted after staining with PE mouse anti-human CD9, BD 555372, PE mouse anti-human CD63, BD 556020, and PE mouse anti-human CD81, BD 555676. The sorted vesicles can then be assessed for PCSA/Ago as above.

[00816] 2) Flow sorting cells and vesicles

[00817] A Beckman Coulter MoFlo™ XDP flow cytometer and cell sorter was used to determine the expression of the indicated proteins on VCaP cells and VCaP vesicles. For cell staining, VCaP cells were detatched and washed in PBS. Approximately 3 x 10^6 cells were resuspended in 1ml Fc Block solution (Innovex Biosciences, part #NB309) and incubated at 4°C for 10 minutes. lOOul aliquots (3 x 10^5 cells) were transferred to staining tubes, washed once in 500µ1wash buffer (eBiosciences, cat # 00-4222) and resuspended in 80-100µ1 PBS-BN (phosphate buffered saline, pH6.4, 1% BSA and 0.05% Na-Azide) and pre-optimized concentration of the indicated antibodies. The antibody/cell solutions were incubated for 30 minutes at 4°C in the dark, washed once in lOOul of PBS-BN, resuspended in 250µ1of PBS-BN and analyzed in the MoFlo analyzer.

[00818] The cytometer was compensated before evaluation using commercially available compensation beads for FITC and PE with Summit Software integrated compensation software. For cells, the Gain for the linear FSC channel was 2.5 with linear SSC having voltage 491 and gain of 1.0, FL1 with voltage 433 and gain of 1.0 and FL2 with voltage 400 and gain of 1.0. For vesicles, the gain for FSC was increased to 3.5, the voltage for FL1 was increased to 501 and the voltage for FL2 was increased to 432 in order to increase detection of the smaller particles.

[00819] The Beckman Coulter MoFlo™ XDP flow cytometer and cell sorter was also used to sort various populations of vesicles in the following manner. Circulating MVs (cMVs) were stained using optimized concentrations of antibodies against the indicated proteins. Positive gates were set using matching isotype control antibodies to define positive and negative regions. The MoFlo sorter was used to isolated positive events using the high-purity sorting mode (i.e., "Purify 1 Drop") to ensure that sorted events were pure to >90%. The MoFlo is capable of sorting two populations at rates of up to 50,000 events per second. For these sorts however, to ensure purity and efficiency of the particle sort, the rate was between 200-300 events per second on average. Subsequent evaluation using an aliquot of the sorted population rerun in the cytometer confirmed >90% purity of the population. Positive events are sorted into 2 ml tubes. The sorted vesicles can be used for further analysis, e.g., miR content within the sorted vesicles can be assessed.

Example 32: Protocol for Immunoprecipitation of Circulating Microvesicles

[00820] This Example provides a protocol for immunoprecipitation of circulating microvesicles (cMVs) from using antibodies to two markers. Any appropriate antibody can be used that will capture the desired vesicle markers of interest. The protocol can further be applied to different sample sources, such as analysis of vesicles

PREOMICS EXHIBIT 1002
Page 1154 of 1861

from various bodily fluids. In this Example, prostate specific vesicles are double immunoprecipitated from plasma using antibodies to PCSA and CD9.

1) Thaw 1 ml plasma from a subject of interest. For example, a subject having prostate cancer or a control, such as a normal male without prostate cancer.
2) Stain the unconcentrated plasma with 40 µĩ anti-PCSA-PE conjugated antibody and 45 µĩ of anti-CD9-FITC to the plasma.
3) Mix and incubate for 30 minutes in the dark at room temperature.
4) Concentrate the plasma using 300kD columns from 1 ml to 300 µĩ to remove unbound antibodies.
5) Remove and set aside 50 µĩ of concentrated plasma to determine the starting content. Save for flow analysis, store 4°C.
6) Add 20 µĩ of anti-FITC microbeads to the remaining 250 µĩ of stained concentrate.
7) Incubate in the dark, refrigerated on a shaker for 30 mins.
8) Prepare MultiSort columns (Miltenyi Biotec Inc., Auburn, CA) by washing the columns with 3 x 100 µĩ washes with Separation Buffer (Miltenyi) off the magnet.
9) After the 30 minute incubation with anti-FITC microbeads (Miltenyi), dilute the stained and labeled plasma by adding 200 µĩ buffer to reduce viscosity. Dilute further if still too thick.
10) Add the -470 µĩ plasma solution to the top of a first washed column, column 1, sitting on the magnet.
11) Allow the plasma solution to flow through.
12) Add 2 x 100 µĩ washes to the upper reservoir to remove un-magnetized particles.
13) Total flow through for column 1 is -670 µĩ. Save for phenotyping.
14) Remove column 1 from the magnet.
15) Add 300 µĩ of buffer and plunge firmly to remove magnetized cMVs from column 1.
16) Add 10 µĩ Multisort Release Reagent (Miltenyi) to the retained volume (300 µĩ).
17) Mix and incubate 10 mins in the dark at 4°C.
18) An optional wash step can be performed to remove released microbeads as necessary.
19) Add 20 µĩ MultiSort Stop Reagent (Miltenyi) to the cMV solution.
20) Add 20 µĩ anti-PE MultiSort Beads (Miltenyi).
21) Mix and incubate 30 mins in the dark at 4°C.
22) Add the solution to the top of a second column, column 2, while on the magnet.
23) Allow to flow through and collect as flow through.
24) Add additional 100 µĩ to wash any un-magnetized particles off column 2 (-450 µĩ).
25) Collect flow through and reserve for flow evaluation.
26) Remove column 2 from the magnet and add 300 µĩ buffer.
27) Plunge firmly to dislodge retained cells, reserve for flow evaluation.
28) Add 10 µĩ of Release Reagent to cleave the beads.
29) Incubate 10 mins in the dark at 4°C.
30) Add 20 µĩ Stop Reagent.
31) Move to flow evaluation.

[00821] Vesicles can also be immunoprecipitated in a sample using a single antibody and column step as desired. For example, prostate specific vesicles can be captured performing a single immunoprecipitation with anti-PSCA antibodies.

[00822] Flow analysis. Five populations collected above are analyzed by flow cytometry: 1) initial unseparated plasma; 2) flow through column 1; 3) retained column 1; 4) flow through column 2; and 5) retained column 2. All populations had CD9-FITC and anti-PCSA-PE added above. Beads were removed but the PE-conjugated antibodies remained on the cMVs and could be evaluated in the flow cytometer.

1) Transfer solutions of cMVs to TruCount tubes for quantification of cMVs/events.
2) Evaluate by flow cytometry using a Beckman Coulter MoFlo-XDP cell sorter.Calculate the number of events based on TruCount tubes (Beckman Coulter).

PREOMICS EXHIBIT 1002
Page 1155 of 1861

[00823] Other markers, such as listed in **Table 5** herein, can be used for vesicle immunoprecipitation using this protocol. For example, vesicles have been immunoprecipitated using one or more of MFG-E8, PCSA, Mammaglobin, SIM2, NK-2R. The immunoprecipitated vesicles can be used for further analysis, e.g., determining vesicle levels or assessing other markers, e.g., surface antigens or payload, associated with the immunoprecipitated vesicles.

Example 33: Normalization of miRNA expression in plasma cMVs to cMV level

[00824] This Example illustrates a method of normalizing miRNA expression in a bodily fluid by combining fluorescence intensity of cell-type specific cMV surface protein markers, immunoprecipitation and nucleic acid detection. This procedure allows for the amplification of a biomarker signal between groups of interest. This Example illustrates this approach to distinguish plasma samples from subjects with prostate cancer and normals (i.e., non-prostate cancer) using miR-22, a microRNA which has been shown to be up-regulated in prostate cancer. See Zhang et al. microRNA-22, downregulated in hepatocellular carcinoma and correlated with prognosis, suppresses cell proliferation and tumourigenicity. *Br J Cancer* **103**:1215-20 (2010).

[00825] Plasma microvesicles from prostate cancer and normal donors were doubly immunoprecipitated with an anti-CD9 antibody (CD9-FITC BD Biosciences Catalog # 555371, BD Pharmingen, San Diego, CA) and an anti-PCSA antibody (prepared in-house) using the approach outlined in **Example 32. FIG. 18A** shows the input plasma for an exemplary sample using a Beckman Coulter MoFlo-XDP cell sorter and flow cytometer to identify positive events. In **FIG. 18A,** it can be seen that whole plasma has mostly double negative events (i.e., CD9-/PCSA-). There are some double positives (i.e., CD9+/PCSA+) in the top right quadrant denoted as R7. Following the double immunoprecipitation comprising capture on a first CD9 column followed by release and a second capture to a second PCSA column, the observed cMV population is significantly enriched in CD9+/PCSA+ double positive events. See **FIG 18B,** which shows the population after the double immunoprecipitation.

[00826] The populations described above were lysed and evaluated for miRNA/niRNA content. The levels of miR-22 in unprocessed plasma were higher in normal than cancer samples. See **FIG. 19A.** A similar trend was observed with miR-22 levels from total RNA isolated from total cMVs in concentrated plasma. See **FIG. 19B.** However, the raw copy number of miR-22 in isolated CD9+/PCSA+ cMVs was higher in the cancer samples compared to non-cancer. See **FIG. 19C.** This separation was enhanced when comparing the number of observed copies of miR-22 from each double positive cMV population to the matched PCSA MFI obtained as in **Example 20** using anti-PCSA as a capture agent. See **FIG. 19D.**

[00827] In a second experiment, a single immunoprecipitation using anti-PCSA antibodies was performed using the above method and the resulting cMV population was evaluated by flow cytometry. The results of flow analysis of an exemplary sample of input material are shown in **FIG. 18C.** There were few PCSA+ events at the outset. Following the immunoprecipitation with anti-PCSA antibodies, the population was strongly enriched for PCSA + cMVs. See **FIG. 18D.** This population was then lysed and evaluated for miRNA/niRNA content from both prostate cancer donor plasma and normal donor plasma. See **FIGs. 19E-19G.** As with the double immunoprecipitation, the separation between cancer and normal was enhanced when comparing the number of observed copies of miR-22 from each PCSA positive cMV population to the matched PCSA MFI.

PREOMICS EXHIBIT 1002
Page 1156 of 1861

Example 34: Score for normalization of antibody captured miR expression to antibody level

[00828] This Example illustrates a method of producing a score to distinguish plasma from cancer patients from non-cancer patients by detecting a level of miRNAs inside circulating microvesicles (cMVs).

[00829] Plasma from prostate cancer patients and normal individuals (i.e., without prostate cancer) was filtered with a 1.2 uM filter then concentrated with a 150 kDa column to concentrate cMVs. See **Example 17.** In order to measure prostate specific cMVs, a PE conjugated anti-PCSA antibody was incubated with 200 µĩ of the concentrate. The PCSA labeled concentrate was purified for PCSA expressing cMV by using a Miltenyi magnetic column. See **Example 32.** RNA from the retained beads containing the PCSA expressing cMV was isolated using Qiagen miRNeasy (Qiagen Inc., Valencia, CA). 60 µĩ of the PCSA labeled concentrate was run on a microsphere assay consisting of HPLC purified antibodies to PCSA, PSMA, B7H3, CD81, CD63 and CD9. The antibodies to PCSA, PSMA and B7H3 were used as capture agents and fluorescently labeled antibodies to CD81, CD63 and CD9 were used as detectors. See **Examples 22-23.** Median fluorescence levels (MFI) were recorded.

[00830] RNA was isolated from each sample concentrate. The copy number of miR-22 and let-7a was determined using Taqman assays with a pre-amp step on an ABI 7900 (Applied Biosystems, life Technologies, Carlsbad, CA). To calculate a diagnostic score, the copy numbers of miR-22 and let-7a in each sample were multiplied by 10 and then divided by the MFI of PCSA in that sample as determined using the microsphere assay. The sum of these values was added to the MFI value of PSMA from the microsphere assay. The average of all three values produces a diagnostic score which was used to differentiate between cancers and normals. In other words, the diagnostic score equals the average of 10*miR22 / PCSA MFI, 10*let-7a / PCSA MFI and PSMA MFI.

[00831] A threshold for the score was determined using 40 randomly selected samples. Using a threshold score of 53 1 or above to distinguish cancer, a performance of 83% sensitivity and 63% specificity was obtained. See **FIGs. 20A** and **20B,** wherein the threshold is indicated by the dashed horizontal line. **FIG. 20C** shows an ROC curve generated with the data. The AUC was 0.77. This threshold was used to classify an independent cohort of 20 samples, resulting in a performance of 82% sensitivity and 67% specificity.

Example 35: miRNA signatures of PCa

[00832] This Example illustrates miRNA signatures of circulating microvesicles (cMVs) that can be used to distinguish prostate cancer.

[00833] Twenty-one of the samples described in **Example 34** that were purified for PCSA expressing cMVs were used to identify microRNA that distinguish the various sample populations. The sample chort comprised eight prostate cancers, three high grade PINs, two inflammatory disease, and six normals (i.e., no prostate conditions). The miR content of the isolated RNA from the PCSA expressing cMVs were analyzed using Exiqon cards as described in **Example 25.** Statistical analysis was performed to identify miRs that significantly differentiate cancer samples. The top 17 miRs included miR- 182, miR-663, miR-155, mirR-125a-5p, miR-548a-5p, miR-628-5p, miR-5 17*, miR-450a, miR-920, hsa-miR-619, miR-1913, miR-224*, miR-502-5p, miR-888, miR-376a, miR-542-5p, miR-30b* and miR-1 179. **FIG. 21** shows illustrative plots for miR-920 **(FIG. 21A)** and miR-450a **(FIG. 21B).** As shown in the figure, miR-920 is overexpressed in confounding diseases whereas miR-450a is down regulated in cancers.

PREOMICS EXHIBIT 1002
Page 1157 of 1861

Example 36: Analysis of Protein, mRNA and microRNA Biomarkers in Circulating Microvesicles (cMVs)

[00834] Vesicles protein biomarkers are analyzed using a microsphere-based system. Selected antibodies to the target proteins of interest are conjugated to differentially addressable microspheres. See, e.g., methodology in **Example 22.** After conjugation, the antibody coated microspheres are washed, blocked by incubation in Starting Block Blocking Buffer in PBS (Catalog # 37538, Thermo Scientific, a division of Thermo Fisher Scientific, Waltham, MA), washed in PBS and incubated with the concentrated cMVs from plasma as described below. Following capture of cMVs, the microsphere-cMV complexes are washed and incubated with phycoerythrin (PE) labeled detector antibodies to the tetraspanins CD9, CD63 and CD81 (i.e., PE labeled anti-CD9, PE labeled anti-CD63, and PE labeled anti-CD81) and washed prior to being detected on the microsphere reader. The fluorescent signal from 100 microspheres is measured and the median fluorescent intensity (MFI) for each differentially addressable microsphere - each corresponding to a different capture antibody - is calculated. Various combinations of detector and capture antibodies are examined in addition to the tetraspanin detectors described above.

[00835] Flow cytometry is used to determine the total number of cMVs in the patient samples. Patient plasma samples are diluted 100 times in PBS then incubated for 15 min at room temperature (RT) in BD Trucount tubes (BD Biosciences, San Jose, CA) for quantification of events per sample. Trucount tubes contain a known number of fluorescent beads that can be used to normalize events for each sample by flow cytometry. Sample acquisition by FACS Canto II cytometer (BD Biosciences) and analysis by FlowJo software (Tree Star, Inc., Ashland, OR) are used to determine the number of sample events and number of Trucount beads per tube. Calculation of absolute number per sample is obtained following manufacturer's instructions (BD Biosciences) and adjustment by dilution factor as necessary.

[00836] MiRNAs are examined from the payload with cMVs from the plasma samples. cMVs are concentrated and the miRNAs are extracted using a modified Trizol method. Briefly, cMVs are treated with Rnase A (20 µg/mĭ for 20 min @ 37°C; Epicentre®, an Illumina® company, Madison, WI) followed by Trizol treatment (750 µĭ of Trizol LS to each 100 µĭ) and vortexed for 30 min at 1400 rpm at room temperature. After centrifugation, the supernatant is collected and RNA is further purified with the miRNeasy 96 purification kit (Qiagen, Inc., Valencia, CA) and stored at -80°C. Forty ng of RNA are reverse transcibed and run on the Exiqon qRT-PCR Human panel I and II on an ABI 7900 (Applied Biosystems, life Technologies, Carlsbad, CA). See, e.g., **Examples 13-14, 25.** C_T values are calculated using SDS 2.4 software (Applied Biosystems). All samples are normalized to inter plate calibrator and RT-PCR control.

[00837] Messenger RNA (mRNA) is also examined in the cMV payload from the plasma samples. cMVs are isolated and treated with RNase A as above. mRNA is extracted using a modified Trizol method as above and purified with a Qiagen RNeasy mini kit precipitating with 70% ethanol (Qiagen, Inc.). The collected RNA is reverse transcribed and Cy-3 labeled using Agilent's "Low Input Quick Amp Labeling" kit for one-color gene expression analysis according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA). Labeled samples are hybridized to Agilent's Whole Genome 44K v2 arrays and washed according to manufacturer's specifications (Agilent Technologies). Arrays are scanned on an Agilent B scanner (Agilent Technologies) and data is extracted with Feature Extractor (Agilent Technologies) software. Extracted data is normalized with a global normalization method and analyzed with GeneSpring GX software (Agilent Technologies).

PREOMICS EXHIBIT 1002
Page 1158 of 1861

[00838] Both miRNA and messenger RNA can be examined from specific subpopulations of cMVs from the plasma. For example, cMVs are concentrated then the population that is positive for PCSA is isolated using immunoprecipitation. See **Examples 32-33.** The PCSA+ cMVs are isolated and miRNA and mRNA is isolated and analyzed as described above. The same methodology is used to examine the miRNA and mRNA content of vesicles isolated using different capture agents directed to different vesicle surface antigens of interest. In addition, the vesicles can be isolated that are positive for more than one surface antigen. See **Examples 32-33.**

[00839] Normalized analyte values are imported into either R (available from The R Project for Statistical Computing at www.r-project.org) or SAS software (SAS Institute Inc., Cary, NC). The data is filtered using appropriate quality control measures and transformed prior to analysis. Analysis is performed as follows:

[00840] Signature performance evaluation (for pre-specified or novel signatures)

[00841] The sample sets generated using the methods above (i.e., payload analysis of isolated vesicle populations) can be used to evaluate the performance of a biosignature that is fully specified prior to either the unblinding of clinical outcome or to the unblinding of clinical laboratory testing of samples. In such a case, the signature is considered *pre-specified* and must be applied, unmodified, to new analyte data on this sample set to obtain predicted outcomes for all samples. Performance of the pre-specified signature is evaluated by comparing predicted and true outcome (for example, in terms of diagnostic sensitivity, specificity, and accuracy). Statistics include performance estimates and confidence intervals.

[00842] For signatures that are not pre-specified (i.e. that are derived with foreknowledge of *both* clinical outcome and laboratory testing results of samples), these samples may still be used to evaluate the performance of the signature. However, to reduce potentially biased estimates of performance, statistical analyses are performed nested within a k-fold cross validation loop that includes marker selection and class prediction steps as described below.

[00843] Marker selection for novel signatures

[00844] Markers are included in novel signatures if they are statistically informative by testing for their association with disease outcome using a subset of commonly applied techniques known to those of skill in the art. These include: 1) Welch test - robust parametric statistical test for difference between group means when variances are unequal; 2) Wilcoxon signed-rank test - robust non-parametric statistical test that can be interpreted as showing an improvement in ROC AUC (above 0.50); 3) Youden's J - calculated as the maximum combined sensitivity and specificity for a marker, across all possible diagnostic thresholds. Statistical significance is evaluated via permutation tests.

[00845] Markers are judged statistically informative if the test is significant in the context of the number statistical tests performed. More specifically, comparison-wise p-values are adjusted for multiple testing - e.g. using false discovery rate thresholds or by control of family-wise error rates.

[00846] Formation of novel signatures

[00847] Once a subset of informative markers is identified in the marker selection stage described above, novel multi-marker models are formed using well-established modeling techniques. Parameters for signatures are estimated by training the models on the full training data set, and performance for the signature is evaluated as described under "Signature performance evaluation" using the approach "for signatures that are not prespecified." Simple and well-established modeling techniques are used in these steps, including: discriminant

PREOMICS EXHIBIT 1002
Page 1159 of 1861

analysis, support vector machines, logistic regression, and decision trees. Results for all models will be reported and optimal markers panels are identified accordingly.

[00848] Additional *aposteriori* analyses are performed on the data set for clinical variables of interest as available. Such variables include age, ethnicity, PSA levels, digital rectal exam (DRE) results, number of previous biopsies, indication for biopsy and biopsy result (e.g. HGPIN, ATYPIA, BPH, prostatitis or prostate cancer), and the like. Such analyses are performed by introducing covariates or stratification variables into previously defined models. P-values are corrected for multiple testing.

Example 37: Biological Pathway Expression in Circulating Microvesicles (cMVs)

[00849] In this Example, expression profiling of mRNA payload in cMVs is performed. Pathway analysis of mRNAs expressed in the cMVs is performed to identify the most significant biological pathways.

[00850] To profile mRNAs in whole vesicle populations, cMVs were isolated from 1 ml of plasma from three prostate cancer and three non-cancer control samples using filtration and concentration as described in **Example 20.** RNA was extracted from 100 µï of plasma concentrate, which was then subdivided into 25 µï aliquots for lysis with Trizol LS (Invitrogen, by life technologies, Carlsbad, CA) after treatment with RNASE A. The aqueous phase from each of the four aliquots was precipitated with 70% ethanol, combined on a single Qiagen mini RNA extraction column (Qiagen, Inc., Valencia, CA), and eluted in a 30 µï volume. The eluted RNA can be difficult to reliably quantify by standard means. Thus, a 10 µï volume was used for the subsequent labeling reactions. Samples were cy-3 labeled with "Low Input Quick Amp Labeling" kit from Agilent for one-color gene expression analysis according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA), with the following modifications: 1) The spike-in mix for Cy3 labeling was altered so that the third dilution was 1:5 and 1 µï was added to each sample; 2) 10 µï of sample was reduced in volume to 2.5 µï using a vacufuge in duplicate for each sample; 3) Every sample was processed in duplicate throughout the protocol until the purification step of the amplified samples. At the beginning of the purification protocol, the duplicate samples were combined and subsequently passed through the column; 4) The samples were not quantified after purification but rather the full volume of the purified sample was hybridized to the array. Labeled samples were then hybridized to Agilent Whole Genome 44K microarrays according to manufacturer's instructions (Agilent Technologies). Data was extracted with Feature Extractor software (Agilent Technologies) and analyzed with GeneSpring GX (Agilent Technologies). 4291 mRNAs were found to be present in the concentrate, including those found in **Table 20.** The GeneSpring software was used to identify pathways that correlated with the expression patterns. Following the above analysis, the androgen receptor (AR) and EGFR1 pathways were the most significantly expressed pathways in the vesicle population. The members of the AR and EGFR1 pathways are shown in **Table 21:**

Table 20: mRNA Expression in Total cMVs

DNAJA1, RPL23, RPS13, VASH1, YWHAZ, ORMDL3, UBE2I, DNTTIP2, RPL18, HLA-DRB 1, C6orf62, GGA1, IMP3, JUN, NUDC, HLA-DRB 5, NDUFB9, BTF3L1, RNF1 1, KLK3, DENND4B, NECAP2, PLAC8, C14orfl66, HNRNPU, MTHFS, TCP1, U2AF1, MRPS12, IL8RBP, OAZ1, STRA13, C2orf79, EBNA1BP2, HMGN1, PYCR2, CREB3L1, CHRDL1, ZNF254, UBE2B, GAPDH, NDUFV2, LCP2, VDAC3, TSSC1, RBM22, YWHAZ, GABARAPL2, PPP1CA, FCNl, DNAJB6, CD44, KIAA0430, HSP90AA1, ATP5J2, C17orf72, GLCCIl, 7-Sep, CTSC, TNRC18, ARL6IP1, HLA-J, GPX4, SYK, RPL23AP53, SDPR, SFRS3, RPL35A, UBC, TALDOl, NKG7, MFN2, TINF2, SNCA, LYN, RHOC, PPIA, RHOA, TPM3, ATP6V1F, MYOIF, MUC5B, HS2ST1, BOLA3, HMGN1, FKBP1A, LOC10013 1582, DNAJA1, RPL10, SYCE1L, RPS25, RPS2, CDKN2A, AHSP, EPB42, C21orf7, LOC100288578, CFD, LOC100134569, LCK, CD52, HSD17B10, OAZ1, MAT2A, DCI, HSPA1A, RPL23A, CCT3, AQP2, LSP1, RNF10, RPL39, EIF3E, RPS29, MLXIPL, KPNA2, UTF1, TALDOl, CRLF3, YWHAB, HBQ1, SSR4, ST13, HLA-DRB3, PFN1, NOS3, FAM102B, WHAMM, PRR13, NPEPL1, MCL1, LOC100132247, NONO, IL26, CCDC69, LBH, RPL35, NCOR2, FBRS, RPS10, RPL4,

PREOMICS EXHIBIT 1002
Page 1160 of 1861

FAM128B, RPS10, FBRSL1, DYNLRB1, ISCU, PLA2G16, PRR5, RNASEH2A, TNRC6B, RPL36, PGLS, LGALS9C, NCOA4, SFRS5, CPNE5, C3AR1, RPL14, EEF1D, EMX1, STK10, RPS10, ZFP36, C21orf58, SPATA2L, MTA1, FLJ43681, MRPS6, HIST1H2AD, PSMD8, ITGB2, RPSA, PMEPA1, PARP1, TRAPPC5, ARPC5L, MRPL41, PDE4C, CCDC108, ANKK1, APBB1IP, MCTS1, TCL1A, HLA-A, ZNF775, POLD4, ACTB, CYBA, DAD1, ARF1, MRPS21, FAM107A, RPL38, SMARCC2, DNAJB2, ANXA1, EVPL, PHPT1, ZNF784, GRB2, SCYL1, VPS4A, RPL23AP7, CTNNB1, HIST1H4H, SMARCC2, RPL36AL, WIPE, VPS35, C10orfl25, RPL10A, RPS15, CARD 16, GPSM3, EIF3C, FPR1, ICT1, BZW1, C15orf28, HLA-A, RAB18, ETFB, IL1B, SLC45A4, BAX, IFI27, PPIA, NYX, SLC27A1, ANXA1 1, ACRBP, TERT, NDUFA6, ZCCHC18, CDC42, RPL30, TNRC4, PWP1, LOC729046, NDUFA4, UFC1, TUB, RDBP, ERBB2, OAZ1, RPS3, TPSG1, HNRNPAl L2, ARMCX6, FAM43B, C16orfl 1, CASP3, MIP, CUTA, PABPC1, LOC283663, HMOX1, RPS10P7, GNAS, C4orf3, MRPS21, SPARC, LSM3, TBCB, GRAMD1C, CHMP4A, RASL10B, LOC100293539, NDUFC1, CWC15, CHRNB2, KRT10, SNX3, RAP1A, CPLX2, ILDR1, HIST1H2BI, ADAMTS13, MRPL34, FKBP3, ZNF680, SRRM3, MYPOP, FTH1, MMD, POLR2F, ODC1, BLOC1S1, UBE2L3, MCM7, C14orfl56, RPSA, ARHGAP1, ATP5SL, SOD1, RANBP1, CARD8, NACA, NCRNA00152, SUM02, H3F3C, SNRPF, YWHAQ, SCLT1, DAD1, SNTA1, DHRS1, CYB5R3, SNX5, SLC25A5P1, ZNF714, C9orfl3 1, MTMR14, RNF44, LOC100132161, HLA-DPB 1, OR10H2, ID2, SSRP1, RPS27, MXI1, TEAD3, LOC648771, TMEM158, TIAM1, RPSA, IFI27L1, HINT1, USP33, H2AFZ, BLOC1S2, TNFAIP8L2, HMGB1L1, C20orfl08, RPS29, LM02, HNRNPAl L2, LOC647121, RAC1, NPC2, SMR3A, HIST1H4B, FXYD5, LARS, RALGDS, NBPF3, THEM5, MAPKAPK3, RPL23, TMSB10, MMP28, C19orf56, HMBS, PSMA2, MTCH1, GNB2L1, COX6B1, UBB, TIMM9, CASP8, BRD7, LCE3E, RPL14, MT1G, LBH, RPL3, RPL13, FLOT2, SYMPK, PMPCB, HMGN2, EEF1D, ROD1, PTP4A2, PCBPl, CACNB3, FHIT, TMBIM6, LCE1D, HRASLS5, TEF, TPT1, RPS15, SNHG5, RPL9, MIER1, MYC, DNAJC4, C6orf25, RPL21, CABP7, CTXN1, STMN1, FAM96B, SELK, COX17, SNRPB, FLJ22184, EIF3B, C12orf65, U2AF1, RPL32, FYN, SP5, LOC100130107, CCDC56, NBPF20, MMADHC, PRDX5, SPINK7, BTN3A2, TMEM38A, ZNF2, DECRl, NDORl, CDK3, HNRNPAl L2, SMAD2, HCN2, TOMM20, PFN1, SFRS18, B2M, SUB1, PKM2, COX6A2, NLGN2, MBD2, RILPL2, CASP1, NACA, CCL5, RPL37A, RPL22, DYNLL1, SAT1, LSM5, LOC441245, ZFAND6, EEF1G, MAP3K3, LSM1, PSMB6, HBG1, EPHX3, HDAC1, LCE5A, PSMC1, MCM3, BAX, MRPL13, TUBA3D, MTIF3, NCF1, RPS17, RPL10, CIRBP, PSMA6, AMICA1, HNRNPA3, RPS25, C19orf56, POLE4, MAP1LC3B, FASTK, RPL23AP82, UQCRC1, RPL24, PRELID1, RPS19, RPL5, PGK1, KIAA0494, HP1BP3, DMWD, RPL26, EIF6, PCBP2, TRMT1 12, SEC1 1A, RPL21, MEI1, CCNI, NCKAP5L, TMSL3, AHNAK, BTF3, HNRNPAl, PTPN6, SIPAILI, POLR2J, C3orfl, C6orf48, LOC10012873 1, PCBPl, C17orf49, ETS2, HIST1H3D, TUBB6, SH3BGRL3, CIAOl, FAM58A, HIST1H2BE, MRPL20, RPL29, HIST3H2A, LOC407835, RPL37, RAB35, FLU, TNFRSF14, FAM129A, GNG5, RPL24, JAK1, C5orf39, LILRB3, C16orf3, A2M, ZNF592, NPHS2, HIGD1A, RP3-377H14.5, KRTAP5-8, PIP5K1C, FAM124A, C22orf32, S100A13, IFITM1, CSDA, NDUFA6, RPL12, FTH1, RARRES3, ZFAND5, RPL29, DAP3, RNF7, COX4I1, FAM1 10A, FOXN3, CXCR4, BBC3, RPS8, CD79A, POTEE, APOL3, PPM1A, FECH, RPLPO, EIF3K, LOC100293090, GGCT, TMEM93, RPS7, RAP lGAP, RABEP1, CEBPB, LGALS3, RCOR2, VIM, IFITM5, Clorfl44, EIF3L, CAPNS1, NBPF10, S100A12, E2F2, COX5B, ZNF24, CTBP2, RABACl, C1 lorf83, ANKDD1A, CD48, HSPB1, VAV1, LSM4, GLTPD1, SH3KBP1, RPL3, RPS2, RPS3A, LCE2A, DAB1, LDHA, CMTM3, MTPN, SCARF2, AES, CD4, LOC645955, PFDN2, ELP2, CTDSP2, LSM6, EIF2B1, METAP2, TRMT1 12, ARPC2, TCF4, APOL1, TRMT5, LOC647979, SLC39A4, RPS15A, EIF3L, WFDC3, EVX1, CHCHD2, ARHGAP25, SNW1, SNHG8, TBCA, KIAA0125, HIST1H4E, ACTB, KLF6, EEF1D, SLC2A1, ACADVL, RPS28, C19orf44, HDAC7, RPSAP52, NDUFA1 1, KIAA0240, CYTH1, GSTOl, MCAT, LAMP1, LOC644950, HIST1H3B, NDUFV1, MKRN1, TUBA4A, RPLPO, PALM, DNAJB5, PLEKHB2, UCRC, CLEC2D, CAMKK2, HMGN4, FAM1 19A, RPL18A, NGDN, RP1 1-431022.2, KIF2A, HBB, SLC25A37, CMTM7, THOC7, ATP5G2, C7orf41, MAFA, VMA21, C14orfl62, CLC, SLC25A5, LOC100132247, MKNK2, LOC729992, EEF1A1, 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PREOMICS EXHIBIT 1002
Page 1161 of 1861

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PREOMICS EXHIBIT 1002
Page 1162 of 1861

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C5orf26, RPL3 1, PPP1R16B, POP4, C16orf53, H3F3A, C21orf33, MESP1, LST1, CALM2, PEX10, PARD6G, SARDH, TAT, HLA-DPB2, RBM27, LOC100288418, LOC10029105 1, SLC35E4, ATG16L2, C3orfl0, TCF3, NR1H3, SNX10, BCAM, NF2, HIST1H1E, LOC100190939, HIST1H2BG, UBE2D3, RPLP1, PLEKH02, TNR, EXOSC8, LOC100133075, RPAP1, FLJ10357, BIRC3, RPL1 1, LOC100292388, RPS2P32, MED 19, ELFN1, TIMM17A, COX7A2L, PSMB9, DDX24, TADA3, SEMA3B, RPL3 1, GSK3A, SYNCRIP, MORF4L1, RPL26L1, AP1S2, FYB, C17orf37, C20orf30, LOC729313, FAM1 19B, CCT8, TSEN54, GABARAPL2, NDUFA8, GPSM3, CIB1, NXT1, C17orf74, CHMP4A, KRT8, CBX3, SLC35B2, DAZAP2, IFI30, BATF, POLD4, LOC100287848, SNX26, EZR, LSM2, CHST13, DDT, EIF3D, ATP5D, GBP6, RPS13, FBX09, STK40, RBP7, HBA2, NDUFAF2, MT1A, H3F3A, ANKS3, LCE1C, MEX3D, SLA, HADHB, TTRAP, SRGN, RHOC, BOLA1, DOK3, GLIPR2, RPS3, LOC100287521, RPLP1, ERP29, RPL17, HCP5, AHNAK, BMP8B, RPS2P45, LOC401859, MVP, CTBP1, RILP, HLA-DRA, LSM12, RPL23AP7, RPL15, HIST1H3C, ARF3, HMGB2, RPS3A, ZNF24, TYK2, FAM36A, EIF3F, SERBP1, COL27A1, EIF1AY, NUDT13, IAH1, ITSN2, RIC8A, C9orf89, LYSMD2, PSMA1, HN1, FLU, ACTR3, TPM4, UBE2D2, BTBD6, BOLA2B, PPA1, P704P, HEBP1, SURF2, PSMA3, HRK, MX1, PTGES3, MUC2, LOC729082, HBD, NAMPT, NSUN5, WIPF1, TYMP, PDCD10, CSNK1E, IER5, MYL12B, CNPY2, PSIP1, NDUFB9, PSMD4, ACTR10, STRAP, C19orf25, EIF4B, HBG1, FRAT2, MKRN1, CDKN1C, ZNF681, MEG3, ZNF646, TBC1D10C, HOMER3, CAPZA1, CALM3, FLJ43681, AGPATI, RPIA, RAX2, DDX5, TAF7, ITPK1, FAM102A, DNTTIP1, RPS14, DCTN3, CA1, COPS5, FUZ, CHURC1, CSNK1G2, NDRG2, CNIH4, FAU, ACBD7, LEF1, SRI, EXOC3L2, CIB1, EBF4, RPL26, TCEAL6, HIST1H3A, LOC1001291 13, HMGN1, DNAJC8, LBX1, FOXC2, HMGB1, POLM, ZNF644, REPS1, C12orf57, TAX1BP1, YBX1, RNF130, NHP2L1, NACA2, PABPC1, MEF2D, RARS2, TSEN34, RPS7P5, NOP58, ZG16B, EIF4B, ATXN7L2, UBE2E3, TPM3, NDUFS6, LOC92659, LZTS2, TUBA4A, CLU, TUBA4A, EEF1G, KIAA1949, SAPS1, FKBP4, NDUFAF3, GLUD1, LGALS1, PRCP, LY86, ERGIC3, AIF1, C3orfl0, ATF4, CXorf40B, FAM108A1, SYN1, SF3B1, ATXN2L, PLAC8, ECHS1, Clorfl62, HSPE1, TUBB2A, TNFAIP2, NBPF3, RRP7B, MRPL38, MYH9, VASP, ALOX5AP, RPL21P44, HLA-F, OTUD5, GRASP, RPS21, SYNGR4, YWHAG, DSTN, ATPlAl, HIST1H4I, SlOOAl 1, Clorf38, TP53TG1, F13A1, DLCl, BAT3, FIBP, HSPA8, Clorfl52, LOC100129122, KLHL35, LOC131055, SORL1, SSR2, CBX7, LOC90499, CITED4, RPL13A, CDC42EP5, BCAP3 1, SEPX1, LYPLAL1, NDUFAB1, ZC3HAV1, PPP1R1 1, PRKCB, TPM1, WNT6, RNPEPLl, SECTMl, NSA2, CDC42SE2, RAB32, LOC100288252, C3orf26, DUSP15, AMZ2, RPL36A, APRT, SCARA5, CSPP1, VAT1, RHOQ, HPS6, BCR, PSMD1, LAS1L, MLXL1, FBXL17, PKM2, HINT1, GYPC, PYY2, LYNX1, SAP 18, ACTC1, FAM107B, HHATL, LCE1A, LOC152217, MRPL21, LOC728723, FIS1, THRAP3, RPS9, CRISPLD2, HEBP2, FCER1G, SPHK2, KRTCAP2, COPE, C10orfl04, C18orf23, PFDN5, HIST1H3F, ENOl, OSBPL8, PTPN18, HNRNPA1L2, IDH3B, ANXA6, TST, RHOA, POMP, RPL10L, TOMM20L, HMGN1, PRELID1, GUK1, PTPRCAP, RPLP0P2, C19orf22, LOC646791, TNFAIP8, LOC100192204, CCM2, LST1, MGST3, COPS6, C17orf89, CNOT2, ABLIMl, HSPA4, ZNF254, RPL34, NDUFS8, GLTSCR2, EIF4A1, FOXN2, SMARCD3, CTSB, EDARADD, ERH, TFF3, UBE2V1, CSDA, MLL5, IKZFl, CCL24, LILRAl, CROCCL2, C20orf24, RFTN1, HSP90AA1, ATP6V1G1, MAPK1 1, FOXOl, HMGN1, FAM45A, HIST1H2AJ, ACTBL2, EVL, TPM1, RASSF5, RRAS, ARHGEF15, NDUFA3, MT1E, HIST1H3E, RPL7, HIST1H2BN, TCOF1, PRAMl, FAM108A1, CASP5, AP1S2, CHCHD1, MT1H, PLEKHJ1, HIST1H4C, MYL12B, FLJ1 1235, PCK2, RAVER 1, TCEAL8, HCST, SUB1, RAB13, FAM162A, ATP5G3, SCNM1,

PREOMICS EXHIBIT 1002
Page 1163 of 1861

ANXA2, LOC100291560, ZNRDl, HLA-E, NDUFA4, MT2A, HIST1H3H, C19orf56, ONLY, ACTGl, WDR82, RANGRF, RASSF2, PHRF1, MON1B, STB, SERBP 1, AHNAK, ARL6IP4, LOC400061, DEXI, PIP4K2A, C6orfl06, ALOX5, JUNB, MEX3D, ZNF56, FAMl 13B, C20orf30, BUB3, EHD1, GLTSCR2, ZFAND5, RPS5, RPL7A, RPL10, SLC8A1, C19orf33, C1lorfl7, DNM1P35, RPL23AP7, HBA1, POLR2L, HLA-G, LOC388564, RHPN1, CNTNAP2, UCN2, HNRNPA2B1, SLC2A4RG, KIAAl 143, UCP3, SNX3, SSTR3, PFDN5, TUBB6, LOC100288578, MAT2A, PGD, CD36, LOC100289383, CDC2L1, RPL7A, H3F3B, EEF1A1, EIF4H, KRTAP2-4, C22orf9, LST1, GNAI2, HIST1H4J, TMEM149, RNASET2, NDUFS7, ZNF91, NOL7, ZNF714, WASF2, DIAPH1, PF4, COMMD1, C20orf24, H3F3A, RAB1B, RPL19, SNRPF, PF4V1, TRAM2, RPL9, ZNF48, RBM14, BRD2, NAMPT, PAIP2, NET1, SND1, TMEM141, PNKD, NOP56, MYL12A, RPL34, ITGB1BP1, NBPF10, EVI2B, PPDPF, EEF1D, GDNF, NBPF15, FOXP1, SARS, TPM3, KIAA1429, FAM49B, GFAP, ISCA1, INPP5K, HMGB1, SLC22A18AS, PPCS, ATP5J, ZNF706, MBNL1, HIST2H2AB, SUPT4H1, NT5C3, C17orf54, R3HDM2, RHOG, EIF1B, UBOX5, LOC391769, SFRS16, DUX4, CAMLG, ARIH2, RIOK3, ARPC3, ZNF625, UBC, DCAF12, LGALS7B, TIPRL, CAMTA1, CHCHD7, RAB7A, FAM108A1, ID1, FAMl 17A, ACTGl, POLR3GL, ARAPl, VCP, ABLIM3, YWHAZ, LOC728324, C17orf79, SERPINBl, CEBPD, YPEL3, BAT4, STB, RPL5, LOC391358, JTB, HIST1H4F, PRDX6, C14orfl69, KRTAP2-4, SNRPD1, LOC730256, STRADB, CHCHD10, TSPAN5, H3F3A, HIST1H2BD, LOC391334, ARHGAP9, HIGD2A, DDAH2, RPL13, TCEB2, TCEA1, ATP5A1, MRPL9, CCT5, SEC61B, FABP5, ACTR3, ZNF738, CDK2AP1, ALDOA, RPS28, BEST4, UQCRB, SIK1, ALDH2, CHCHD2, LIMD2, LAT2, C7orf47, CTSS, TOMM7, BBC3, CD48, PARP10, RAB10, FGD3, ARHGEF10L, ZNF92, NPIPL3, RPL29P2, FTL, GPX1, CASC3, MEA1, RPL31, LOC729991-MEF2B, ZNF341, RNF 113A, MYCBP2, RPA4, FAM13 1B, TES, USP10, CECR1, CNFN, GNG10, DUSP6, CNIH, PRDX1, ATPIF1, GALR3, PARK7, LSM10, CMTM6, TXN, KLF2, NSUN5C, LOC100294102, PSMA6, SH3BGRL, BAALC, MALATl, PABPC3, LOC100128775, CDA, RPL6, MRPL52, MAPRE2, SECISBP2, 13-Sep, TUBA1C, HMGB1, SNRPC, RSL1D1, HIST1H3B, LOC4403 11, AMD1, N4BP2L2, FTHL17, DLGAP3, SLC25A5, HES7, hCG_20426, PRDX2, GDF15, RSU1, FZD9, RWDD1, CLTC, PRPF40A, MED29, C2CD4A, EFHD2, TUSC2, PTP4A2, BECN1, DCAF1 1, LGALS7, PSMC1, EFNB3, TRAK2, TMEM63B, FKBP2, TAF12, SCAF1, ZNF727, CYB5R3, RPL18, PSMD9, IFITM4P, LOC729406, RBX1, POLR2J2, RPSA, DCAF4L2, GTF3A, ERCC2, USP15, LOC100131482, ACTR3, GIMAP4, EIF3K, HIST2H2AC, FCGR3A, TRAPPC2L, WFDCIOB, TRAK2, HIST1H2AC, COX7A2, MRPL53, GSTOl, LOC646890, MAF, FLJ35390, VCP, VAMP8, HIST1H3D, CSDC2, SAT2, RPL21, PDZKIIPl, UBE2R2, Clorfl 13, CD63, TPM3, BNIP3L, PPIAL4A, HSPBl, RSL1D1, UGP2, RPL27, GPR153, ACAT2, NCAPH, BRI3, WASH2P, PTP4A3, C19orf33, FYB, SNX2, ROBLD3, RPL19P12, HDAC2, LSM3, SERF2, CCT2, APBB1IP, PAX4, RPLP2, LSM8, MDH1, FGD1, DDX54, MAGOHB, ZNF182, SLC4A1, NCRNA001 16, SF3B5, CCT6A, HIST1H2BE, CFLP1, EEF1G, HIST1H2BB, CLNS1A, PABPN1, RPA1, LOC100292427, SNF8, SAP 18, IL27, SLC34A3, UQCR, NDUFS5, PLAC4, ZCCHC17, ZDHHC22, HIST1H4D, NBR1, C1lorf2, ALDOA, HMBOX1, DRD4, RAC2, C6orf62, NDOR1, ROBLD3, ATAD3C, NOP10, SSR2, CCDC88B, GTF2H5, EIF4A2, C10orf84, RPS7, DYNLT1, RPS4X, CT47A1 1, IER2, NUDT21, LOC100128355, CHP, RPS25, CAT, SMCR5, PSMC6, PDCD4, 7-Sep, HSD17B1 1, VDAC1, TUBA8, COX4I1, CLEC2D, PPIAL4A, LOC92249, PLIN4, HBA2, NENF, C19orf53, C17orf91, HLA-DMB, CDC42SE1, TMEM14B, CASC3, SNRPG, PRRB, TBX21, SLC44A2, IL7R, SrVAl, CDV3, DHX9, LOC728741, TMEM9B, CSDE1, IFITM2, ALKBH7, TSPYL2, PHB2, ORC6L, TRA2B, EMG1, GSN, OCIAD2, SIGIRR, ATP6V1A, WDR6, LFNG, GNG10, SNRPD3, HSBP1, LIME1, H3F3B, COMMD4, RNF126, ACTGl, CEACAM19, MT1X, COPZ1, HNRNPA1L2, TXNDC17, EIF3F, RPS4Y1, MFNG, CLTA, CCDC57, SPRR2E, FAM65A, AP3S1, UBXN1, RDBP, NSA2, SP1 10, TXNIP, RNF5, HLA-E, SDHAF1, LOC100129616, UTS2R, PTGES3, RPSA, COX7B, CEBPA, NME1, EID1, STARD8, PRKACB, ATN1, ADA, HNRNPA3, YBX1, URM1, FBXL19, PGK1, CNBP, AURKAIP1, C22orfB, RPL3, EIF3E, RAP IB, FBL, VDAC2, C19orf29, PPID, FAM89B, ARHGEF1, APEH, PCMTD1, CKS1B, RPL13, UQCRH, LOC100129292, POMC, TCF25, PLEKHF1, YWHAZ, RPS10, HIST1H2AJ, ERN1, HNRNPK, NRGN, YWHAZ, PTMA, PDCD6, LAPTM5, LYZ, C1QBP, FDPS, RPS15A, EXOC7, OSTF1, HIST1H2BF, FAM13 1C, EEF2, HIST1H2AM, MRPL33, SOX1, C4orf48, PRB3, NME2P1, CD37, CTDSP1, COX8A, FAM96A, RASGRP2, RP1 1-9412.2, TAOK2, TAF1L, HLA-DPB 1, TRIM58, STK4, HSPA8, LOC100288418, UQCRFS1, C7orf28B, SMARCE1, EPAS1, C19orf38, HIST1H4K, EIF3I, STK17B, CDKN1B, ISG15, NDRG1, C20orfl41, EEF1A1, RPSA, CCDC1 15, NKX2-8, RPL13AP3, PPIA, SUSD3, ATP5J2, ZNF100, C6orfl, C7orf28A, CGGBP1, FLOT1, HSF1, KLF16, WAC, SCLT1, AMD1, UBXN6, UBE2F, SEC13, SSBP1, ZDHHC4, SERF2, RPS6, LRRC2, ENOl, ANXA2, CYTH4, RHOA

Table 21: Pathway Expression in Total cMVs

Pathway	Members
Androgen Receptor (AR)	GTF2F1, CTNNB1, PTEN, APPL1, GAPDH, CDC37, PNRC1, AES, UXT, RAN, PA2G4, JUN, BAG1, UBE2I, HDAC1, COX5B, NCOR2, STUB1, HIPK3, PXN, NCOA4
EGFR1	RALBP1, SH3BGRL, RBBP7, REPS1, SNRPD2, CEBPB, APPL1, MAP3K3, EEF1A1, GRB2, RAC1, SNCA, MAP2K3, CEBPA, CDC42, SH3KBP1, CBL, PTPN6, YWHAB, FOXO1, JAK1, KRT8, RALGDS, SMAD2, VAV1, NDUFA13, PRKCB1, MYC, JUN, RFXANK, HDAC1, HIST3H3, PEBP1, PXN, TNIP1, PKN2

[00851] In a related set of experiments, expression profiling was performed in PCSA+ cMVs. PCSA+ cMVs were isolated using immunoprecipitation as in **Example 32.** Expression was performed as above using Agilent

PREOMICS EXHIBIT 1002
Page 1164 of 1861

Whole Genome 44K microarrays. 2402 mRNAs were found in the PCSA captured samples, including those shown in **Table 22.** The TNF-alpha pathway was the most significantly overexpressed pathway. The members of the TNF-alpha pathway are shown in **Table 23.**

Table 22: mRNAs Expression in PCSA+ cMVs

LOC100132006, EHMT2, RPL23, RPS13, HDDC3, LOC150759, MEGF1 1, CRCP, LRP1, CDH6, C9orf30, MAB21L2, EPHA2, SYT2, BOK, NLE1, C2orf53, ORMDL3, TUT1, CYP2E1, C6orf81, GATAD2A, RPL18, C3orf21, MASP1, CLOCK, CENPP, NFKBIB, LOC729915, C17orf64, BLK, NR1H2, SMARCA4, ACVRL1, ARFIP1, NFKB2, tcag7.1 196, JUN, LOC100128760, ZFYVE19, HLA-DRB5, NDUFB9, MFN1, CLK1, LOC100289600, SULT1A2, BTF3L1, IGSF10, RALY, KLK3, KIAA1751, RUVBL1, DNAJC3, C14orfl66, BCAP3 1, HNRNPU, DFNB3 1, CENPBD1, WNK2, PNLIPRP1, OAZl, GEMIN5, C19orf55, CHRDL1, CACNA1B, LOXL4, HIRIP3, hCG_1643808, COX4I2, FABP3, GAPDH, TSSC1, GABARAPL2, EXOl, POU6F2, STX8, HSP90AA1, TNFRSF1 IB, SPACA5, TNRC18, SAP30BP, FOXK2, RPL23AP53, PIK3R4, XPC, SOCS7, RPL35A, UBC, NKG7, SNCA, PTK2B, PPIA, LOC100289350, CDKN2B, TNNI2, MUC5B, MED6, NOL8, HS2ST1, SNRPC, LOC10013 1582, DNAJA1, RPL10, RPL10, SYCE1L, RPS25, PNMALl, RPS2, CDKN2A, LOC100288578, CFD, LOC100134569, CD52, NLRC5, OAZl, DCI, RPL23A, LSPl, RPL39, VTA1, RPS29, MLXIPL, FAM133B, UTF1, HBQ1, SSR4, HLA-DRB3, NOS3, LICAM, WHAMM, PRR13, IGF1, GPR61, IL26, CCDC69, RPL35, TFE3, RPL4, FAM128B, PAK1IP1, RPS10, FBRSL1, MEIS1, DYNLRB1, ISCU, PRR5, FCGR2A, DNAI2, EPSTI1, TNRC6B, RPL36, NCOA4, XRN1, RPL14, EEF1D, RPS10, LOC388152, TRIM55, SPATA2L, FLJ43681, TSSC4, HIST1H2AD, JMJD6, TCF7L2, RPSA, PMEPA1, PRR13, SIRPG, ANKK1, MCTS1, TCL1A, HLA-A, ALDH3B1, POLD4, ACTB, SDK1, RPL38, SEC62, CASZ1, EVPL, ZNF784, DEPDC6, ZNF638, RPL23AP7, COMMD7, SMARCC2, RPL36AL, PHF21A, RPL10A, OR2H1, ATAD3A, C10orf82, RPS15, CAP2, CARD 16, ZDHHC16, FPR1, ETFB, NPPC, BAX, IFI27, NYX, SLC27A1, LOC100133280, CAPN10, CI lorf67, ACRBP, TERT, NDUFA6, RPL30, TNRC4, NDUFA4, ERBB2, OAZl, RPS3, TPSG1, FAM43B, C16orfl 1, SIRPA, PABPC1, FAM73B, HMOX1, RPS10P7, GNAS, HPD, NEXN, SPARC, RASL10B, LOC100293539, TMTC4, C21or©l, GMPR, ADAMTS13, VDR, SRRM3, MYPOP, FTH1, BLOC1S1, UBE2L3, APP, RPSA, SOD1, CARD8, NACA, NCRNA00152, HTR1F, SUM02, PHLDB3, H3F3C, PVALB, LOC286272, SCLT1, C9orfl3 1, MTMR14, RNF44, HLA-DPB 1, TP63, FGD3, RPS27, LOC648771, TMEM158, POLD3, RPSA, PEX1, USP33, POLRMT, RPS29, FXYD5, RALGDS, BTRC, ZCCHC6, THEM5, RPL23, VAX2, TMSB10, MMP28, HMBS, GNB2L1, COX6B1, UBB, CASP8, CCS, LCE3E, RPL14, MRPL38, RPL3, RPL13, SYMPK, EEF1D, DNMT3A, PCBP1, FHIT, LCE1D, KCNQ1DN, HRASLS5, SYT15, LOC643668, TPT1, RPS15, SNHG5, RPL9, DNAJC4, MYCNOS, RPL21, EGLN3, TUBG1, SNRPB, FLJ22184, RPL32, ASCC3, ZNF2, HCN2, B2M, PKM2, COX6A2, NACA, CCL5, RPL37A, PRM3, TTC1, DYNLL1, EEF1G, ACOT13, PSMB6, HBG1, LCE5A, BAX, LOC284998, RPS17, RPL10, TRIM24, IMP5, RPS25, RPL23AP82, SMARCA2, RPL24, PRELID1, RPS19, RPL26, TRMT1 12, RPL21, LOC100129917, CCNI, TMSL3, TMEM140, C6orf70, CA5A, POLR2J, C6orf48, LOC10012873 1, DLAT, PCBP1, MIA2, REEP5, SH3BGRL3, RPL29, HIST3H2A, RPL37, THUMPD1, APLNR, LOC10028833 1, SEC14L2, EXOC1, LPIN3, ZNF592, NPHS2, FANCM, RP3-377H14.5, FAM124A, S100A13, IFITM1, RPL12, IGF2, FTH1, RPL29, MAP3K6, CDC2L1, ING5, BBC3, RPS8, CD79A, LOC100129291, RPLPO, EIF3K, LOC100293090, RPS7, RABEPl, RAB8A, RCOR2, LOC2201 15, VIM, IFITM5, NBPFIO, S100A12, COX5B, PPP1R14A, CD48, HSPB1, GLTPD1, RPL3, RPS2, RPS3A, LCE2A, CLIP3, DAB1, MTPN, SCARF2, CD4, IL12RB2, ARPC2, RPS15A, EVX1, C19orf71, YWHAE, SNHG8, TBCA, KIAA0125, HIST1H4E, ACTB, EEF1D, TNNT2, RPS28, ZNF761, HDAC7, RPSAP52, PRICKLE3, BAG5, LOC644950, PHLDA1, RPLPO, UCRC, PALMD, RPL18A, HBB, GRIN3B, ATP5G2, MAFA, C14orfl62, EEF1A1, SLC25A6, FAU, NDUFS7, SLK, COL6A2, SYNPO, RPL23A, UBA52, MYL6, COMMD6, HOXA3, GPR132, PLEKHN1, BST1, SURF1, CDC2L5, RPS16, ADAMTS7, MON1B, C10orf47, POLR2A, RPSA, TAOK3, ZC3H6, HIST1H1C, RPS2P32, RPS27A, RPL18A, MEX3D, POLR2E, 5-Sep, RPS20, SHISA9, ALX1, RPS4P16, MIF, RPS26, PAX9, LOC64203 1, LOCI 00289600, SF3A2, CCDC88C, RPL14, USMG5, RPL17, VPS 18, PFKFB3, KCNK15, LOC728449, RPS3A, TOMM7, ALAS2, ZBTB46, GRIN2D, RPL8, NEDD8, FAHD1, KLHL34, IK, GMFG, GNAZ, GOSR1, SEPW1, SCRT2, LOC100288165, CCDC24, C16orf81, UBL5, RYBP, TNRC18, NKX1-2, SUPT4H1, ATP51, RPLPO, EN2, BZRAP1, SDK2, RAB8A, RPL34, MXD4, FAM63B, UBE2S, ATP5D, MRGPRF, CD99, BAG1, POLD1, POLR2L, CDC2L1, RAC2, SIRT3, NPM1, RPS2, DDX49, LOC649294, URG4, TAPT1, EIFl, MAN1B1, ARHGEF1 1, ATP6V0C, Clorfl75, PLEKHOl, HCLS1, LSMD1, NFE2L1, CI lorflO, UBC, LEPROT, CEL, HIST1H1D, SFRS8, HIST2H2AC, RPL37A, MED13L, PPIAL4A, FCRL2, RPL35, KRTAP1-3, LOC440461, S100A6, SOD2, PAX6, SAPS1, FAM129B, LIPE, NME2, FLJ23867, KLF14, RPS1 1, HCN4, PAX8, CSTA, ZNF713, POTEF, RPS14, FTL, FOXD3, MXD1, RPL35, HYAL1, ZNF506, ATF6B, CRIPl, ZNF467, IFI16, RABGAPIL, AN07, CGB1, PTRF, LOC10013 1262, PPBP, SNX19, PTMA, HBM, LOC341056, SERPINB1, RPS10, C17orf88, CFL1, RPL23A, TUBA1C, VDAC3, HSPA8, TP53TG3, RGS10, BCAM, PFDN6, EEF1D, TMEM201, PABPC1, OGDH, RPL34, NNAT, RSRC2, LOC730144, RPL27A, ZNHIT1, TALDOl, FOXQ1, LRRC6, BTF3, ARL6IP4, MEPE, C15orf21, LENG8, N4BP3, LGR4, GJC1, FAM128B, IRX5, A4GALT, CTSA, PPIA, RALBP1, TAF3, LOC541471, MMP1 1, ADAMTS10, IL1 1, C9orfl67, FLJ3 1356, RTN3, NIN, RYR3, YLPM1, PPIAL4A, OR2H1, RPS3A, LOC389641, FAM177A1, MAGEE1, CYP2W1, HEMGN, ZFPMl, HIST1H2BO, RRAS, RPS3A, EIF3M, PTMA, EIF3D, TAGLN2, LMODl, CASP4, GZMB, NGFRAPl, RPS17, AGAP3, SOX4, RPS5, NDUFB2, PCMTDl, EIFl, C19orf73, SIKl, PSMBIO, ROMOl, CD86, RPS10, FAM154A, HILS1, NCOA4, ATP5L, LOC100288418, MDFI, EEF1B2, A2ML1, OSMR, DLX1, SOD1, SEC62, RPL12, LOC10013033 1, RNF15 1, VSIG10L, NDUFA1, RPS26, JMJD8, LOC283 177, LTA4H, CYP2F1, DIRCl, Clorf27, TRIM10, SUM02, HLA-B, GH1, VAMP2, TNXB, LOC100288755, SUPV3L1, SPEF1, tcag7.907, RPS19, HIST3H2BB, RSPH10B, TBX3, RPS10, UQCRQ, hCG_19809, RPL13A, ZFR2, RHOBTB1, RPL21, MAX, CAMKK2, PDZD7, DCAF10, FTL, DEFA3, IQSEC2, NRG4, GIPR, GNAS, RELB, ITIH2, CASP4, S100A4, OGFR, RPLP0P3, CCDC93, HMGB1, RPL36A, PPIA, NDUFA1, TUBA4A, TUBA IB, SCD5, HIST2H2AA4, SAFB, PTMS, BLVRB, HIST1H2AM,

PREOMICS EXHIBIT 1002
Page 1165 of 1861

FTH1, RPL29, STEAP4, RPL15, TMIGD2, LTB, APOC4, HNRNPA1, SLC12A7, HLA-DOB, RPL9, GGT6, ZNF525, SLC8A2, EN1, PKN2, KRT16, ODF3L2, BAX, NFE2L1, CCDC50, TPT1, CKB, IFITM3, LOC39985 1, UXT, NAB2, DYNLL1, TUBBl, SOX3, RPL21, GUK1, CHD7, RPS2P32, GCET2, GRAP, SPSB4, GSTP1, UBE2G2, HIST1H2AH, VCX3A, SERGEF, DUSP9, SMEK1, FCRLB, ECHDC2, MTA1, SAFB, CREB3L3, LOCI 19358, DRAP1, TMEM175, RPL21, RPL21P44, LRP3, FAU, MOGAT1, DYRK4, TYROBP, RPL22L1, C12orf40, PTGR2, SLC46A1, LCE1F, MORN1, PALM2-AKAP2, COX6C, RPL41, ALOX15B, MYL6, RGS18, CI lorB 1, C18orf32, CUL1, ARHGDIB, MPPED1, NPM1, ATP5E, LOC648771, TNXB, HIST1H2AE, ACTB, CCDC62, RPS26, H2AFJ, TMSB4X, LOC15 1009, RPL30, RPL23AP71, HIST2H3D, BID, AGRP, RPL28, ZNF219, FXYD5, GPR149, MGC10814, RPL39, GZMH, GPR150, PTMA, RPS3A, CEND1, CYB561D1, POU5F1P4, UBB, SPPL2B, CLDN5, GBP4, HLA-B, CASKIN1, TMSL3, ZNF746, NFYA, VGLL2, ODAM, ADAMTSL5, DPEP3, PSPH, ALPPL2, GRN, NPAS3, SLAIN2, ADRA2C, NPM1, C14orf2, COX6A1, PRNP, ATP5E, RPL7, PRR24, CDCA3, CXorfl8, HNRNPA1, RPL10A, HIST1H2BH, BCLAF1, hCG_2014417, TMEM30B, GNAZ, RPL7A, ANAPC1 1, C21orf81, PRKCG, TMED10P, C6orf25, S100A9, NDUFB10, WNT2B, 9-Sep, ACTB, CRTC1, ZNF157, SSX2, WNT10A, EHD2, SSTR2, RPS23, HSPB1, RHO, TMEM1 11, GSTK1, LOC100132247, C16orf93, XRCC6, CCNTl, NDUFA2, NDUFB4, RPS14, CTTN, RPL34, TMEM191B, H2AFJ, TRIM29, EEF1D, UBA52, HLA-C, LOC729732, EXOC3L2, CCDC72, HBD, EDF1, UBA52, PTMA, VPS28, AES, NDOR1, IER2, C2orfl4, SMARCA4, ZNF575, LRFN1, SRP14, RPL21, UBC, LPXN, TMIE, HIST1H2AK, ID4, RPL10, RPL17, PRR7, CHCHD2, SLX5, RPS7, TIMM8B, MT2A, LOC100130152, FABP6, SCNN1D, MTPN, TPT1, RUVBL1, RPL7A, HLA-H, IL32, EIF3C, GNG1 1, CACNA1I, PSME1, RPS10, MLL3, HCFC1R1, SNRNP70, FGF3, RSRC1, MSRA, PHOX2A, VIM, GAS 5, HIST2H3D, C20orfl99, HIST1H4L, H3F3B, HIST1H2BL, ZNF658, TMEM86B, LDHB, RPL23A, LOC100290566, ZNF579, EEF1D, RPL12, H3F3A, FBL, DBI, ARPC5, LGALS2, LIG3, CI lorf58, EIF3H, HOXB13, NRN1, RAB31, ASAH2, EDNRB, RPL26L1, RPL22, CACNB2, AP3D1, ATP50, TNXB, EEF1A1, RPL21, ATP 5I, HMGN2, CALM3, ISG20, NCRNA00188, DCAF8, CXorfl8, HIST3H3, CDKAL1, LOC338864, C17orf96, RPS18, COX7C, LCP1, TSPAN10, FTH1, RPS13, VARS2, RPS27, FTSJ2, RPL6, PSME2, RPL29, SMPD4, Clorfl62, LTB4R2, YWHAZ, FOXS1, MAX, RPS12, SOX1 1, ITGAX, YBX1, SLC25A3, HLA-DPA1, FTH1, RPL21, KCNAB3, SSX5, UTP14A, EEF1B2, RPL35, HLA-E, RPL37, KRT77, RPL13, PSMB1, RPL21, S100A8, RPS24, RPS27A, HIST1H2BK, RPL34, CDH22, RUFY1, BTF3, GUCA1B, IFI27L2, MYEOV2, RPL13A, NCRNA00181, NACC1, LOC344967, CA2, ACAD10, SNRPD2, LCE1A, LOC90246, NPM2, H3F3A, MESP1, LST1, CALM2, CASZ1, PEX10, LRRC18, PARD6G, TAT, RBM27, LOC100288418, LOC10029105 1, SLC35E4, C3orfl0, PIAS1, BCAM, FAM55A, LOC100190939, RPLP1, RPL1 1, LOC100292388, RPS2P32, ELFN1, SEMA3B, RPL3 1, ADAMTS13, PHF10, LOC729141, DIDOl, C17orf74, TRPS1, SLC35B2, IFI30, POLD4, LOC100287848, CYSLTR1, SNX26, EIF3D, GBP6, RPS13, HBA2, MT1A, H3F3A, LCE1C, MEX3D, RPS3, KIAA0368, RPLP1, ERP29, RPL17, BMP8B, LOC401859, RPS3A, BOLA2B, P704P, HRK, MUC2, HBD, MYL12B, NDUFB9, HBG1, MKRN1, TBC1D10C, FLJ43681, AGPATI, RAX2, PAK6, FLYWCH2, RPS14, FUZ, ACTR8, TTC17, LPPR5, FAU, ACBD7, EXOC3L2, RPL26, HIST1H3A, DDN, LBX1, FOXC2, C12orf57, LOC642826, NACA2, PABPC1, RPS7P5, ATXN7L2, LOC92659, TUBA4A, TUBA4A, EEF1G, FKBP4, NDUFAF3, LGALS1, AIF1, ATF4, SYN1, RALYL, ATXN2L, C4orf3 1, MRPL38, RPL21P44, OTUD5, ADAMTSL5, GRASP, RPS21, POM121L8P, PLEKHA6, Clorf38, HSPA8, LOC100129122, KLHL35, TCF25, ZNF365, RPL13A, CDC42EP5, WNT6, RNPEPLl, LOC100288252, DUSP15, RPL36A, MIXLl, C19orf77, FBXL17, KLHL22, HINTl, LYNXl, HHATL, BARHL2, MRPL21, RPS9, FCER1G, C18orf23, PFDN5, RPL10L, TOMM20L, HMGN1, GUK1, PTPRCAP, RPLP0P2, LST1, ZNF254, RPL34, ERH, CCL24, CROCCL2, PDE6C, DDX3 1, NDUFA3, FAM71B, HIST1H2BN, ZC3H13, PCDH17, MT1H, ETV5, HIST1H4C, MYL12B, FLJ1 1235, PCK2, RAVER 1, HCST, SCNM1, ANXA2, LOC100291560, NDUFA4, MT2A, KIF19, PELI3, ACTG1, MON1B, BANP, ARL6IP4, MEX3D, VPS 13D, GLTSCR2, RPS5, RPL7A, RPL10, SLC8A1, DNM1P35, RPL23AP7, HBA1, POLR2L, HLA-G, TNFAIP8L3, PTHLH, TOE1, RHPN1, UCN2, UCP3, PFDN5, LOC100288578, IRGC, LOC100289383, RPL7A, EEF1A1, KRTAP2-4, GSTT1, FAM178A, RNASET2, GATS, PF4, H3F3A, RPL19, TRAM2, RPL9, NET1, MYL12A, RPL34, ORAI1, CCDC1 1, PPDPF, EEF1D, GDNF, TPM3, C20orfl5 1, OAS3, AZU1, SLC22A18AS, HIST2H2AB, C17orf54, DPP6, R3HDM2, TSPAN33, C20orf201, LOC391769, SFRS16, DUX4, ARPC3, UBC, LGALS7B, TCOF1, PGM5, ACTG1, YPEL3, NR2C2AP, RPL5, PRDX6, C14orfl69, HCG18, H3F3A, LOC391334, CHST10, MAP6D1, RPL13, C6orfl82, TCEB2, MPHOSPH8, FABP5, ZNF48, ALDOA, RPS28, KCNQ4, GCGR, UQCRB, SIK1, DNMT1, PPAN, TOMM7, PARP10, CDC34, RPL29P2, FTL, GPX1, RPL31, FAM13 1B, CNFN, GALR3, TXN, BAALC, ALKBH2, CDA, RPL6, MRPS22, LOC4403 11, FTHL17, DLGAP3, HES7, FZD9, RWDD1, ANKRD50, SCAMP5, CT47B1, GATA3, DCAF1 1, JAK3, GRM4, LGALS7, EFNB3, SCAF1, ZNF727, PLEKHG4B, GTF2H1, CYB5R3, RPL18, LOC100134359, PSMD9, RBX1, POLR2J2, RPSA, DCAF4L2, FAM163A, RSHL1, IGFBP3, EIF3K, HIST2H2AC, WFDCIOB, LOC10013081 1, USP30, COX7A2, MAF, VAMP 8, CSDC2, RPL21, Clorfl 13, SGK269, BNIP3L, PPIAL4A, RPL27, GPR153, NCAPH, BRI3, NDUFAF2, WASH2P, NID2, C19orf33, RHBG, RPL19P12, SERF2, PAX4, PPARGCIB, RPLP2, LOC440181, FGDl, C15orf37, ZNF182, SLC4A1, GOLGA6L10, SF3B5, HIST1H2BE, EEF1G, MITF, LOC100292427, UQCR, NDUFS5, ZDHHC22, DRD4, RAC2, NDOR1, SSR2, C10orf84, PHF7, RPS7, RPS4X, USP6, LOC440330, DRAMl, SNX15, RPS25, TUBA8, COX4I1, LOC92249, MYBL1, PLIN4, HBA2, C17orf91, TMOD4, PRR13, CDV3, CSDE1, IFITM2, TAF1, SCTR, PHB2, MYH6, FUZ, TCEAL7, LIME1, IL21, MAN1C1, H3F3B, Clorfl 70, GGT1, CEACAM19, HNRNPA1L2, ZFAND2B, ZNF467, UBXN1, OSBPL10, RGNEF, SP1 10, HLA-E, AZI2, SDHAF1, MAPK13, H1FNT, DAP, C16orf82, RPSA, SPRN, STARD8, ATN1, TPTE2, RPL3, EIF3E, FBL, ALPL, DKK4, ARHGEF1, TNFRSF25, PCMTD1, C21orf93, RPL13, UQCRH, LOC100129292, SKIL, WHSC1L1, RPS10, EDEM2, NRGN, IFT57, FOXA3, RUNDC2C, PTMA, NRXN1, TCEA3, RPS15A, FAM167B, EXOC7, HIST1H2BF, HMGA2, FAM13 1C, SIRPD, HIST1H2AM, AIG1, TTLL5, FTCD, SOX1, ZNF138, PRB3, LOC346329, NME2P1, CTDSP1, C14orfl26, COX8A, LOC26102, PSCA, TAF1L, HLA-DPB 1, MYST4, ORC3L, SSX9, HSPA8, RGS12, LOC100288418, HEY2, PCSK4, SOBP, TMEM232, RGS19, ATP2B2, NTN1, C10orf35, PI4KAP2, LOC1002871 14, Clorf95, HADH, C20orfl41, DSCR4, SEMG2, EEF1A1, SCRT1, CYB5A, RPSA, KIN, ST3GAL4, NKX2-8, RPL13AP3, FGF17, PPIA, SAA2, SLC37A1, MYST4, DDA1, PDE1C, CGGBP1, SLC23A3, KBTBD5, FLOT1, HSF1, BAT2D1, KLF16, AMHR2, WAC, EPB41L4A, ETFDH, TNN, SLURP1, CELA3B,

PREOMICS EXHIBIT 1002
Page 1166 of 1861

LOC100272216, KLF1, TRPC4, D21S2091E, RBBP8, SSBP1, KCNH6, GRK4, FIBCDl, SERF2, RPS6, LRRC2, ENOl, DHDDS, Clorf226

Table 23: Pathway Expression in PCSA+ cMVs

Pathway	Members
TNF-alpha	BCL3, SMARCE1, RPS11, CDC37, RPL6, RPL8, PAPOLA, PSMC1, CASP3, AKT2, MAP3K7IP2, POLR2L, TRADD, SMARCA4, HIST3H3, GNB2L1, PSMD1, PEBP1, HSPB1, TNIP1, RPS13, ZFAND5, YWHAQ, COMMD1, COPS3, POLR1D, SMARCC2, MAP3K3, BIRC3, UBE2D2, HDAC2, CASP8, MCM7, PSMD7, YWHAG, NFKBIA, CAST, YWHAB, G3BP2, PSMD13, FBL, RELB, YWHAZ, SKP1, UBE2D3, PDCD2, HSP90AA1, HDAC1, KPNA2, RPL30, GTF2I, PFDN2

[00852] The genes in **Table 24** were all significantly downregulated in PCSA+ cMVs as compared to the total cMV population. Expression was compared using a t-test with Benjamini and Hochberg false-discovery rate correction. Significantly differentially expressed mRNAs are shown in the table (corrected p-value ≤ 0.05).

Table 24: mRNAs downregulated in PCSA+ cMVs compared to total cMVs

RPL23, RPS13, RPL18, NDUFB9, BTF3L1, KLK3, C14orfl66, OAZ1, GAPDH, GABARAPL2, HSP90AA1, TNRC18, RPL23AP53, RPL35A, UBC, NKG7, SNCA, PPIA, HS2ST1, RPLIO, SYCE1L, RPS25, RPS2, CD52, OAZ1, DCI, RPL23A, LSP1, RPL39, RPS29, HBQ1, SSR4, WHAMM, RPL35, RPL4, FAM128B, RPSIO, FBRSL1, ISCU, PRR5, RPL36, NCOA4, RPL14, EEF1D, RPSIO, HIST1H2AD, RPSA, PMEPA1, ANKK1, TCL1A, POLD4, ACTB, RPL38, ZNF784, RPL23AP7, SMARCC2, RPL36AL, RPL10A, RPS15, IFI27, NYX, SLC27A1, NDUFA6, RPL30, NDUFA4, OAZ1, RPS3, TPSG1, PABPC1, HMOX1, RPS10P7, GNAS, LOC100293539, MYPOP, FTH1, BLOC1S1, RPSA, SOD1, NACA, SUM02, H3F3C, HLA-DPB 1, RPS27, LOC648771, TMEM158, RPSA, RPS29, RALGDS, RPL23, TMSB10, GNB2L1, COX6B1, UBB, CASP8, RPL14, RPL3, RPL13, PCBP1, FHIT, LCE1D, HRASLS5, TPTl, RPS15, SNHG5, RPL9, RPL21, FLJ22184, RPL32, ZNF2, HCN2, COX6A2, NACA, RPL37A, DYNLLl, EEF1G, HBG1, LCE5A, RPS17, RPLIO, RPS25, RPL23AP82, RPL24, PRELID1, RPS19, RPL26, TRMT1 12, RPL21, CCNI, TMSL3, C6orf48, PCBP1, SH3BGRL3, RPL29, HIST3H2A, RPL37, RP3-377H14.5, IFITM1, RPL12, FTH1, RPL29, BBC3, RPS8, RPLPO, EIF3K, RPS7, RCOR2, VIM, IFITM5, NBPF10, S100A12, COX5B, CD48, HSPB1, GLTPD1, RPL3, RPS2, RPS3A, MTPN, ARPC2, RPS15A, EVX1, SNHG8, TBCA, HIST1H4E, ACTB, EEF1D, RPS28, RPSAP52, LOC644950, RPLPO, UCRC, RPL18A, HBB, ATP5G2, EEF1A1, SLC25A6, FAU, NDUFS7, RPL23A, UBA52, MYL6, COMMD6, HOXA3, RPS16, ADAMTS7, RPSA, ZC3H6, HIST1H1C, RPS2P32, RPS27A, RPL18A, MEX3D, RPS20, RPS4P16, MIF, RPS26, LOC64203 1, SF3A2, RPL14, USMG5, RPL17, VPS 18, KCNK15, LOC728449, RPS3A, TOMM7, ALAS2, GRIN2D, RPL8, NEDD8, GMFG, SEPW1, LOC100288165, C16orf81, UBL5, NKX1-2, ATP5i, RPLPO, SDK2, RPL34, UBE2S, ATP5D, BAG1, POLD1, POLR2L, CDC2L1, RAC2, NPM1, RPS2, LOC649294, EIF1, ATP6V0C, PLEKHOl, HCLS1, LSMD1, NFE2L1, C1lorflO, UBC, HIST1H1D, HIST2H2AC, RPL37A, PPIAL4A, RPL35, KRTAP1-3, S100A6, SOD2, SAPS1, FAM129B, NME2, FLJ23867, RPS1 1, HCN4, CSTA, ZNF713, POTEF, RPS14, FTL, FOXD3, RPL35, CRIPl, ZNF467, PTMA, HBM, SERPINB1, RPSIO, CFL1, RPL23A, TUBA1C, HSPA8, RGS10, BCAM, EEF1D, TMEM201, PABPC1, OGDH, RPL34, LOC730144, RPL27A, ZNHIT1, TALDOl, FOXQ1, BTF3, ARL6IP4, C15orf21, LGR4, FAM128B, IRX5, PPIA, RTN3, PPIAL4A, RPS3A, MAGEE1, ZFPM1, HIST1H2BO, RRAS, RPS3A, EIF3M, PTMA, EIF3D, TAGLN2, CASP4, GZMB, RPS17, AGAP3, RPS5, NDUFB2, PCMTD1, EIF1, C19orf73, PSMB10, ROMOl, CD86, RPSIO, NCOA4, ATP5L, EEF1B2, SOD1, RPL12, LOC10013033 1, VSIG10L, NDUFA1, RPS26, LTA4H, SUM02, HLA-B, LOC100288755, RPS19, RPSIO, UQCRQ, hCG_19809, RPL13A, RPL21, PDZD7, FTL, DEFA3, IQSEC2, GIPR, GNAS, CASP4, S100A4, OGFR, RPLP0P3, HMGB1, RPL36A, PPIA, NDUFA1, TUBA4A, TUBA IB, HIST2H2AA4, BLVRB, FTH1, RPL29, RPL15, LTB, HNRNPA1, RPL9, GGT6, ZNF525, PKN2, NFE2L1, TPTl, CKB, IFITM3, UXT, DYNLLl, SOX3, RPL21, GUKl, RPS2P32, GSTPl, HIST1H2AH, DUSP9, FCRLB, LOCI 19358, DRAPl, TMEM175, RPL21, RPL21P44, FAU, TYROBP, RPL22L1, LCE1F, PALM2-AKAP2, COX6C, RPL41, MYL6, C1lorf3 1, ARHGDIB, NPM1, ATP5E, LOC648771, TNXB, HIST1H2AE, ACTB, RPS26, H2AFJ, TMSB4X, RPL30, RPL23AP71, HIST2H3D, BID, RPL28, ZNF219, FXYD5, MGC10814, RPL39, GPR150, PTMA, RPS3A, CYB561D1, UBB, SPPL2B, HLA-B, CASKIN1, TMSL3, ZNF746, DPEP3, PSPH, NPAS3, NPM1, C14orf2, COX6A1, ATP5E, RPL7, PRR24, HNRNPA1, RPL10A, HIST1H2BH, RPL7A, ANAPC1 1, S100A9, NDUFB10, 9-Sep, ACTB, CRTC1, RPS23, HSPB1, RHO, XRCC6, NDUFA2, NDUFB4, RPS14, RPL34, TMEM191B, EEF1D, UBA52, HLA-C, EXOC3L2, CCDC72, HBD, EDFl, UBA52, PTMA, VPS28, IER2, SMARCA4, SRP14, RPL21, UBC, HIST1H2AK, RPLIO, RPL17, PRR7, CHCHD2, RPS7, TIMM8B, MT2A, LOC100130152, MTPN, TPTl, RPL7A, IL32, EIF3C, CACNAII, PSMEl, RPSIO, MLL3, FGF3, PHOX2A, VIM, GAS5, HIST2H3D, C20orfl99, HIST1H4L, H3F3B, HIST1H2BL, LDHB, RPL23A, ZNF579, EEF1D, RPL12, H3F3A, FBL, DBI, ARPC5, LGALS2, C1lorf58, EIF3H, RPL22, AP3D1, ATP50, EEF1A1, RPL21, ATP5i, HMGN2, ISG20, NCRNA00188, HIST3H3, CDKAL1, C17orf96, RPS18, COX7C, LCP1, TSPAN10, FTH1, RPS13, RPS27, RPL6, PSME2, RPL29, Clorfl62, YWHAZ, RPS12, YBX1, SLC25A3, HLA-DPA1, FTH1, RPL21, EEF1B2, RPL35, HLA-E, RPL37, RPL13, PSMB1, RPL21, S100A8, RPS24, RPS27A, HIST1H2BK, RPL34, RUFY1, BTF3, IFI27L2, MYEOV2, RPL13A, NACC1, SNRPD2, LCE1A, H3F3A, MESP1, LST1, CALM2, PARD6G, LOC100291051, SLC35E4, C3orfl0, BCAM, RPLP1, RPL1 1, LOC100292388, RPS2P32, ELFN1, RPL3 1, C17orf74, IFI30, POLD4,

PREOMICS EXHIBIT 1002
Page 1167 of 1861

LOC100287848, SNX26, EIF3D, GBP6, RPS13, HBA2, MT1A, H3F3A, RPS3, RPLP1, RPL17, LOC401859, RPS3A, BOLA2B, P704P, HRK, HBD, MYL12B, NDUFB9, HBG1, MKRN1, TBC1D10C, FLJ43681, RAX2, RPS14, FAU, ACBD7, RPL26, HIST1H3A, LBX1, C12orf57, NACA2, PABPC1, RPS7P5, TUBA4A, TUBA4A, EEF1G, NDUFAF3, LGALS1, AIF1, ATF4, ATXN2L, MRPL38, RPL21P44, RPS21, ClorB8, HSPA8, LOC100129122, RPL13A, CDC42EP5, WNT6, LOC100288252, RPL36A, HINT1, LYNX1, MRPL21, RPS9, FCER1G, C18orf23, PFDN5, RPL10L, HMGN1, GUK1, RPLP0P2, LST1, ZNF254, RPL34, NDUFA3, MT1H, HIST1H4C, MYL12B, RAVER 1, HCST, ANXA2, LOC100291560, NDUFA4, MT2A, ACTG1, ARL6IP4, MEX3D, GLTSCR2, RPS5, RPL7A, RPL10, DNM1P35, HBA1, POLR2L, HLA-G, UCP3, PFDN5, RPL7A, EEF1A1, KRTAP2-4, H3F3A, RPL19, RPL9, NET1, MYL12A, RPL34, PPDPF, EEF1D, GDNF, TPM3, HIST2H2AB, R3HDM2, LOC391769, SFRS16, DUX4, ARPC3, UBC, ACTG1, RPL5, PRDX6, H3F3A, LOC391334, RPL13, TCEB2, ALDOA, RPS28, UQCRB, SIK1, TOMM7, PARP10, RPL29P2, FTL, GPX1, RPL3 1, GALR3, TXN, BAALC, RPL6, LOC44031 1, DLGAP3, HES7, ZNF727, RPL18, PSMD9, POLR2J2, RPSA, EIF3K, HIST2H2AC, COX7A2, VAMP8, RPL21, Clorf1 13, BNIP3L, PPIAL4A, RPL27, GPR153, BRI3, WASH2P, RPL19P12, SERF2, PAX4, RPLP2, SF3B5, HIST1H2BE, EEF1G, UQCR, NDUFS5, DRD4, RAC2, NDOR1, SSR2, RPS7, RPS4X, RPS25, COX4I1, HBA2, PRR13, CDV3, CSDE1, IFITM2, PHB2, H3F3B, HNRNPA1L2, UBXN1, HLA-E, RPSA, RPL3, EIF3E, FBL, RPL13, UQCRH, LOC100129292, RPS10, PTMA, RPS15A, HIST1H2BF, FAM13 1C, HIST1H2AM, SOX1, NME2P1, CTDSP1, COX8A, HLA-DPB 1, HSPA8, EEF1A1, RPSA, NKX2-8, RPL13AP3, PPIA, KLF16, SERF2, RPS6, LRRC2

Example 38: Microarray Profiling of mRNA from Circulating Microvesicles (cMVs)

[00853] Large scale screening on high density arrays or mRNA levels within cMVs can be hindered by sample quantity and quality. A protocol was developed to allow robust analysis of cMV payload mRNAs that distinguish prostate cancer from normals.

[00854] cMVs were isolated from 1 ml of plasma from four prostate cancer and four non-cancer control samples using filtration and concentration as described in **Example 20.** RNA was extracted from 100 µï of plasma concentrate, which was then subdivided into 25 µï aliquots for lysis with Trizol LS (Invitrogen, by life technologies, Carlsbad, CA) after treatment with RNASE A. The aqueous phase from each of the four aliquots was precipitated with 70% ethanol, combined on a single Qiagen mini RNA extraction column (Qiagen, Inc., Valencia, CA), and eluted in a 30 µï volume. The eluted RNA can be difficult to reliably quantify by standard means. Thus, a 10 µï volume was used for the subsequent labeling reactions. Samples were cy-3 labeled with "Low Input Quick Amp Labeling" kit from Agilent for one-color gene expression analysis according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA), with the following modifications: 1) The spike-in mix for Cy3 labeling was altered so that the third dilution was 1:5 and 1 µï was added to each sample; 2) 10 µï of sample was reduced in volume to 2.5 µï using a vacufuge in duplicate for each sample; 3) Every sample was processed in duplicate throughout the protocol until the purification step of the amplified samples. At the beginning of the purification protocol, the duplicate samples were combined and subsequently passed through the column; 4) The samples were not quantified after purification but rather the full volume of the purified sample was hybridized to the array. Labeled samples were then hybridized to Agilent Whole Genome 44K microarrays according to manufacturer's instructions (Agilent Technologies). Data was extracted with Feature Extractor software (Agilent Technologies) and analyzed with GeneSpring GX (Agilent Technologies). Genes with expression in at least 50% of the samples were included in the final analysis. 2155 probes were detected that met these criteria. Of these 2155, 24 were found to have significantly different expression (p value < 0.05) between the prostate cancer group and the control group. See **Table 25** and **FIG. 22. Table 25** shows 24 genes that were significantly differently expressed between the mRNA payload from cMVs in the four prostate cancer patient samples and four healthy control samples. **FIG. 22** shows dot plots of raw background subtracted fluorescence values of selected genes from the microarray: **FIG. 22A** shows A2ML1; **FIG. 22B** shows GABARAPL2; **FIG. 22C** shows PTMA; **FIG. 22D** shows RABAC1; **FIG. 22E** shows SOX1; **FIG. 22F** shows ETFB.

PREOMICS EXHIBIT 1002
Page 1168 of 1861

Table 25: Differentially expressed mRNAs in cMVs from PCa and healthy samples

GeneSymbol	p-value	Change in normal	FCAbsolute
A2ML1	0.001	down	1.88
GABARAPL2	0.002	up	1.36
PTMA	0.002	up	1.76
ETFB	0.003	up	1.16
RPL22	0.008	down	1.36
GUK1	0.009	up	1.28
PRDX5	0.011	up	1.48
HIST1H3B	0.014	up	1.29
RABAC1	0.022	up	1.33
PTMA	0.024	up	1.65
C1orf162	0.026	down	1.35
HLA-A	0.031	up	1.23
SEPW1	0.033	up	1.31
SOX1	0.034	down	1.38
EIF3C	0.034	down	1.30
GZMH	0.037	up	1.81
CSDA	0.040	up	1.79
SAP18	0.040	down	1.36
BAX	0.043	up	1.20
RABGAP1L	0.045	up	2.19
C10orf47	0.047	down	1.58
HSP90AA1	0.047	up	1.46
PTMA	0.048	up	1.52
NRGN	0.049	up	2.57

[00855] Abbreviations in **Table 25:** "GeneSymbol" references nomenclature available for each gene feature on the array. Details for each gene are available from Agilent (www.chem.agilent.com) or the HUGO database (www.genenames.org). "FCAbsolute" shows absolute fold-change in mRNA levels detected between groups.

Example 39: Circulating Microvesicle Assay for Ovarian Cancer

[00856] In this Example, the vesicle ovarian cancer test is a microsphere based immunoassay for the detection of a set of protein biomarkers present on the vesicles from plasma of patients with ovarian cancer. The test employs antibodies or other ligand or binding agent (e.g., aptamer, peptides, peptid-nucleic acid) with binding specificity to the following protein biomarkers: CD95, CD9, CD59, CD63, CD81, and EpCAM. After capture of the vesicles by antibody (or other binding agent) coated microspheres to CD95 and EpCAM, phycoerythrin-labeled antibodies are used for the detection of general vesicle biomarkers (here CD9, CD59, CD63, and/or CD81). Depending on the level of binding of these antibodies to the vesicles from a patient's plasma a determination of the presence or absence of ovarian cancer is made.

[00857] Vesicles are isolated as described above, e.g., in **Example 20.** The profiling for such protein biomarkers can itself represent a diagnostic, prognostic or theranostic readout, by comparing the profile in a test sample to that of a reference sample. The reference sample can be a level of microvesicles in a normal sample without cancer, wherein an elevated level of vesicles comprising CD95, CD9, CD59, CD63, CD81, and EpCAM indicates the presence of ovarian cancer.

[00858] In addition, the biomarkers are used to profile, identify or isolate a particular test sample that can be further interrogated for additional biomarkers that may be present in or associated with the microvesicle population. For example, the input sample of microvesicles is subjected to an affinity or immunoprecipitation

PREOMICS EXHIBIT 1002
Page 1169 of 1861

step using a binding agent specific to a biomarker (here, substrate-bound antibody binding CD95 and/or EpCam), and the isolated biomarker-positive (BM+) subpopulation is further processed using methods disclosed herein or known in the art to characterize and determine the presence of additional biomarkers (e.g., proteins, peptides, RNA, DNA) present in the subpopulation of microvesicles.

[00859] The test can further comprises assessing levels of microRNA within the captured vesicles, using methodology presented herein, e.g., in **Examples 13-16.** The microRNA comprises members of the miR200 family, including miR-200c. Decreased levels of the miR200 microRNA as compared to a non-cancer reference indicate the presence of ovarian cancer. Lower levels of miR200 further indicate a more aggressive cancer.

Example 40: miRs differentially expressed in PCa

[00860] Attempts to find a blood-based biomarker for prostate cancer (PCa) detection have been challenging. Quantification of microRNAs (miRs) in blood was used to identify potential genetic biomarkers. Using plasma-derived circulating microvesicles (cMV) as an enriched source of miRs from cells, this Example illustrates a miR biosignature that can distinguish PCa samples from healthy controls as well as a miR biosignature for metastatic PCa.

[00861] A panel of plasma samples from men with prostate cancers and controls (men biopsy confirmed without prostate cancer) were analyzed using Exiqon RT-PCR panels as described herein. Using the TNM scale, the prostate cancers included MX samples (did not evaluate distant metastasis), M0 samples (no distant metastasis), and M1 samples (confirmed distant metastasis).

[00862] miRs were detected in vesicles isolated from the patient samples. RNA was isolated from 150 µĩ of frozen plasma concentrate from each sample using a modified Qiagen miRneasy protocol (Qiagen GmbH, Germany). The modified protocol included treating the concentrated samples with Rnase A before isolation so that only RNA protected within vesicles was analyzed in each sample. The samples were spiked with a known quantity of *C. elegans* microRNA for normalization in subsequent steps. 40 ng of RNA isolated from vesicles in the sample was used for each Exiqon panel.

[00863] The Exiqon RT-PCR panel consisted of two 384 cards covering 750 miRs and control assays. The qRT-PCR assay was performed using a Sybr green assay run on an ABI 7900 (Life Technologies Corporation, Carlsbad, California). Ct values for each miR assay were normalized to the Ct values of inter-plate calibrator (IPC) probes and RT-PCR controls. Several quality checks were put into place. Samples were eliminated from analysis when IPC Ct values were >25, RT-PCR Ct values were >35 and when samples did not amplify control miRs (i.e., miR-16 and miR-21). Principal component analysis of the sample data was performed using GeneSpring software (Agilent Technologies, Inc., Santa Clara, CA) to identify outliers. Three samples were eliminated from the analysis due for failing to qualify using these quality measures.

[00864] Data was subjected to a paired t-test between sample groups as specified below and p-values were corrected with a Benjamini and Hochberg false-discovery rate test. miRs showing the most significant p-values were validated using a Taqman probe approach.

[00865] Ten of 750 miRs compared in non-metastatic PCa (n=64) and normal control (n=28) samples were found to have a >2.0-fold change with a *P* value <0.0 1. See **Table 26.** In a validation set (N=l 68), expression of hsa-miR-107 (P=0.03) and hsa-miR-574-3p (P=0.02) were examined. Both were significantly different between the non-metastatic PCa (n=133) and control (n=35) samples.

Table 26: Non-metastatic prostate cancer vs control

PREOMICS EXHIBIT 1002
Page 1170 of 1861

miR	p-value	Fold Change in Prostate Cancer
hsa-miR-574-3p	0.003	3.32
hsa-miR-141	0.008	3.22
hsa-miR-432	0.002	4.15
hsa-miR-326	0.001	6.36
hsa-miR-2110	0.005	5.98
hsa-miR-181a-2*	0.004	-2.75
hsa-miR-107	0.000	13.16
hsa-miR-301a	0.006	5.41
hsa-miR-484	0.009	2.92
hsa-miR-625*	0.003	4.00

[00866] Comparison of metastatic (n=15) and non-metastatic (n=55) samples found that 16 out of 750 miRs had a >2.0-fold change with a *P* value <0.01. See **Table 27.** Quantitation of these miRs in a subsequent validation set (39 metastatic and 73 nonmetastatic) found that several miRs tested by qRT-PCR were able to distinguish metastatic and non-metastatic PCa (hsa-miR-200b, hsa-miR-375, hsa-miR-141, hsa-mir-33 1-3p, hsa-miR-181a, and hsa-miR-574-3p). In a separate cohort, hsa-miR-141 and hsa-miR-375 levels were significantly higher in cMV from the serum of metastatic PCa patients (n=47) than in cMV of non-recurrent PCa patients (n=72; P=0.0001). See **FIG. 23.**

Table 27: Metastatic vs Non-Metastatic Prostate Cancer

miR	p-value	Fold Change in Metastatic
hsa-miR-582-3p	0.001	2.51
hsa-miR-20a*	0.002	3.62
hsa-miR-375	0.003	10.71
hsa-miR-200b	0.003	3.90
hsa-miR-379	0.005	2.10
hsa-miR-572	0.005	-7.39
hsa-miR-513a-5p	0.005	2.23
hsa-miR-577	0.005	5.90
hsa-miR-23a*	0.005	2.30
hsa-miR-1236	0.005	2.63
hsa-miR-609	0.006	2.31
hsa-miR-17*	0.006	4.80
hsa-miR-130b	0.007	6.12
hsa-miR-619	0.008	3.37
hsa-miR-624*	0.009	6.09
hsa-miR-198	0.009	2.12

[00867] Blood-derived cMVs are a source of miR biomarkers for characterizing a phenotype. miRs biosignatures were able to distinguish non-metastatic PCa blood samples from controls. In metastatic plasma-derived cMV samples, there was higher expression of 7 miRs, and 2 of these (hsa-miR-141 and hsa-miR-375) were further verified to be elevated in metastatic serum-derived cMV. This Example provides blood-based miR biosignatures of cMV for the detection of prostate cancer and identification of metastatic cases.

PREOMICS EXHIBIT 1002
Page 1171 of 1861

Example 41: Isolating Subpopulations of Exosomes and Subsequent miR Profiles

[00868] In this Example, microRNA (miR) expression patterns were examined in circulating microvesicle subpopulations that were defined based on surface protein composition. Vesicles isolated from a prostate cancer cell line (VCaP) were flow sorted based on their surface protein composition using methodology as described herein. The vesicles were evaluated for differential expression of miRs. Phycoerythrin-labeled antibodies targeting EpCam, CD63, or B7-H3 were used to sort the subpopulations of vesicles by fluorescence-activated cell sorting. Vesicles were sorted on a Beckman-Coulter MoFlo XDP (Beckman Coulter, Inc., Brea, CA) so that each vesicle could be analyzed as an individual particle. There was a significant shift in the intensity of the FL2 channel over the isotype control due to the abundance of the antigen on the surface of the vesicles. The sorted subpopulations of vesicles were subsequently profiled by miR expression. The miR profiles for the EpCam, CD63, and B7-H3 positive subpopulations were compared to the profile of the total VCaP vesicle population. Differential miR expression patterns were observed across the subpopulations and all expression patterns were distinct from that observed in the total population. Patterns of both over- and under-expression of miRs were observed between groups. These data show that subpopulations of vesicles can be distinguished and separated based on surface protein markers as well as their genetic content, in this case miRs. The ability to isolate tissue-specific vesicle populations from patient plasma based on surface protein composition and then analyze them based on both surface protein composition and genetic content can be used for diagnostic, prognostic, and theranostic applications as described herein.

Example 42: MicroRNA miR-497 for Detecting Lung Cancer

[00869] There is currently no blood test for the early diagnosis of lung cancer. MicroRNA was examined in circulating microvesicles (cMVs) isolated from plasma samples. Vesicles were isolated as described in **Example 20.** RNA was extracted from the vesicles contained in 1 ml of plasma using a Trizol method. MicroRNA payload was detected using quantitative Taqman® RT-PCR methodology. The expression of miR-497 was examined in plasma from 16 lung cancer patients and 15 control normal adults (i.e., no lung cancer). A significant difference in the copy number of miR-497 was observed between the two groups ($p = 0.0001$). See **FIG. 24A.** Using a threshold of 1154 copies of miR-497 (in 0.1 ml of plasma) to differentiate lung cancer versus normal samples (indicated by the vertical line in **FIG. 24A),** lung cancer was detected with 88% sensitivity and 80% specificity.

[00870] In a follow on study, circulating microvesicles (cMVs) from 24 non-small cell lung cancer (NSCLC) patients of primarily early stage disease (Stage IA = 9, IB = 9, IIA = 1, IIB = 2, III = 1, IV = 2) and 26 healthy individuals were isolated from 1 ml of frozen plasma. The expression of miR-497 was examined in the cMVs from plasma samples from the lung cancer patients and 26 control normal adults (i.e., no lung cancer). Patient characteristics are shown in **Table 28.**

Table 28: Patient Characteristics

Stage	Males	Females
Stage IA	5	4
Stage IB	4	5
Stage IIA	1	0
Stage IIB	1	1

PREOMICS EXHIBIT 1002
Page 1172 of 1861

Stage III	1	0
Stage IV	0	2
Normal	14	12

[00871] Median normalized copy number was 9000±307 copies per ml (±95% CIM) for normal individuals and 27,500±1298 copies per ml (±95% CIM) for patients with NSCLC. Setting a threshold for cancer of 1570 copies in 0.1 ml samples (i.e., 15,700 copies per ml), the assay had a sensitivity of 79% and specificity of 81% and an AUC of 0.89. See results in **FIGs. 24B-24C** and **Table 29. Table 29** shows test performance using cut off thresholds of 13,560 and 15,700 copies / ml. The threshold can be adjusted to favor sensitivity or specificity.

Table 29: miR-497 to Detect of Lung Cancer

True Positive	21	19
True Negative	18	21
False Positive	8	5
False Negative	3	5
Sensitivity	88%	79%
Specificity	69%	81%
Accuracy	78%	80%
AUC	0.89	0.89
Cut off (copies / ml)	13,560	15,700

Example 43: Prospective analysis of a Circulating Biomarker diagnostic assay

[00872] Introduction: With the exception of non-melanoma skin cancer, prostate cancer is the most common cancer affecting American men. In the United Stated in 2010, there were 217,730 new cases of prostate cancer and 32,050 deaths (source is the National Cancer Institute). The clinical behavior of prostate cancer ranges from a microscopic well-differentiated tumor to an aggressive cancer with a high likelihood of invasion and metastasis.

[00873] Despite the significant contribution that the Prostate Specific Antigen (PSA) test has made to the effective management of prostate cancer, it has been widely recognized as having significant shortcomings which result from the antigen being specific for prostate tissue and not for prostate cancer. A normal PSA value is currently considered to be less than 4.0 ng/niL, however, this cutoff remains controversial. If the serum PSA is in the range of 4.0 to 10 ng/niL there is an approximate 30% chance of finding prostate cancer on prostate biopsy even through the use of repeated biopsies (e.g., 10-12 cores). A PSA cutoff of 2.5 ng/mL for prostate biopsy has been recommended in the National Comprehensive Cancer Network (NCCN) guidelines. The American Cancer Society guidelines recommend considering a biopsy if the PSA is higher than 2.5 ng/mL.

[00874] Because the test is highly specific for the PSA antigen, it is elevated in both prostate cancer and in non-malignant conditions such as benign prostatic hyperplasia (BPH) and prostatitis. Furthermore, not all prostate cancers release excessive levels of PSA into the serum and PSA levels can be influenced by a variety of other factors such as concomitant medications, age, and race (e.g., African Americans often have relatively higher PSA levels; Asian men often have relatively lower PSA levels). An elevated PSA, therefore, is associated with

PREOMICS EXHIBIT 1002
Page 1173 of 1861

suboptimal clinical sensitivity, specificity, and positive predictive value as a risk assessment aid in the setting of possible prostate cancer. There is a need for a test to aid in the diagnosis of prostate cancer that adds specificity to the current diagnostic algorithm.

[00875] Biology of Microvesicles: Microvesicles can be created intracellularly by a variety of cell types when a segment of the cell membrane spontaneously invaginates, undergoes exocytosis, and is released into the extracellular environment. A variety of cell types may produce microvesicles including dendritic cells, tumor cells, lymphoid cells, mesothelial cells, epithelial cells, and cells from different tissues or organs. A microvesicle may include any membrane-bound particle that is derived from either the plasma membrane or an internal membrane and is subsequently released into the extracellular environment. Microvesicles are cell-derived structures bounded by a lipid bilayer membrane arising from herniated evagination (blebbing), separation and sealing of portions of the plasma membrane, or from the export of any intracellular membrane-bounded vesicular structure containing various membrane-associated proteins of cellular origin. These may include surface-bound molecules derived from the host circulation that bind selectively to a tumor-derived protein as well as molecules contained in the microvesicle or exosome lumen such as tumor-derived miRNAs, mRNAs, and intracellular proteins. Microvesicles can also include membrane fragments. Valadi *et al.* (2007) profiled the composition and contents of microvesicles derived from mast cells and found them to be substantially enriched in microRNAs (miRNAs, miRs) and messenger RNAs (mRNAs). In addition, the profile of RNAs found in microvesicles was different from the profile of RNAs found in the cytosol. For example, microvesicles contained no ribosomal RNAs, but they contained large numbers of 19-22 nucleotide miRNAs. **FIGs. 25A-C** show a transmission electron micrograph of microvesicles isolated by ultracentrifugation from a prostate cancer cell line grown in culture. **FIG. 25A** is an electron micrograph of Vcap-derived microvesicles bound to a glass slide, **FIG. 25B** is a scanning electron micrograph of Vcap-derived microvesicles, and **FIG. 25C** is a scanning electron micrograph of Vcap microvesicles bound to a polystyrene bead coated with poly-L-lysine.

[00876] The secretion of microvesicles by tumor cells and their implication in the transport of proteins and nucleic acids (e.g. miRNAs) demonstrate a role for microvesicles in the pathological processes. Microvesicles have been found in a number of body fluids including, but not limited to, blood plasma, bronchoalveolar lavage fluid, and urine. Among other biological functions, microvesicles take part in intercellular communication as well as serving as transport vehicles for proteins, RNAs, DNAs, viruses, and prions.

[00877] Microvesicles and Biomarkers: Protein biomarkers or tumor markers comprise protein molecules occurring in blood or tissue that are associated with cancer and whose measurement or identification is useful in disease diagnosis or clinical management.

[00878] Tumor markers can be used for a number of clinical purposes, including without limitation the following: (1) Screening a healthy population or a high risk population for the presence of cancer, (2) As an aid in making a diagnosis of cancer or of a specific type of cancer, (3) As an aid in determining prognosis, and 4) To support treatment monitoring.

[00879] Research on biomarkers that support clinical decisions is increasing rapidly, and in the last few years, a variety of *genetic* biomarkers have been discovered and validated for use in guiding therapeutic decisions for oncology patients (e.g., KRAS in patients with colorectal cancer, Her2-neu over-expression in patients with breast cancer, and EGFR in patients with non-small cell lung cancer). Many *protein* biomarkers available today,

PREOMICS EXHIBIT 1002
Page 1174 of 1861

however, fail to demonstrate the sensitivity, specificity, and predictive value needed by clinicians to support decision-making. The carcinoembryonic antigen (CEA), for example, is a protein that was first identified in patients with colorectal cancer, but has since been found to be elevated in a variety of malignancies including pancreatic, gastric, lung, and breast as well as a variety of benign conditions such as hepatic cirrhosis, inflammatory bowel disease, chronic lung disease, and pancreatitis. Another example is CA-125, an antigen present on 80% of non-mucinous ovarian carcinomas, but it also may be elevated in other malignancies such as endometrial, pancreatic, lung, breast, and colon as well as a variety of benign conditions such as menstruation, pregnancy, and endometriosis.

[00880] Microvesicles produced by tumor cells contain molecules of tumor-cell origin such as miRNAs, mRNAs, and proteins. The isolation and concentration of circulating microvesicles (cMV) can, therefore, present a highly concentrated source of tumor-associated biomarkers.

[00881] This Example illustrates a protocol for developing a microvesicle-based diagnostic test that is characterized by a biomarker signature (biosignature) to provide improved assessment of presence and/or the risk of prostate cancer in men between the age of 40 and 75. This microvesicle-based approach may further be used for staging/monitoring of neoplastic disease, supporting therapeutic decision-making, and determining prognosis.

[00882] As described herein, the invention provides in part a multiplex sandwich immunoassay (described below) based on captured circulating microvesicles that is capable of distinguishing plasma from men with organ confined prostate cancer from men without prostate cancer. This assay comprises a microsphere-based immunoassay for the detection of a set of protein biomarkers present on the microvesicles in plasma from patients with prostate cancer. A number of microvesicle surface antigens were examined during assay development and an optimized biosignature panel or markers was selected. This preliminary approach employed specific antibodies to the following protein biomarkers: CD9, CD63, CD81, PCSA, B7H3 and PSMA.

[00883] Both PSMA and PCSA are prostate specific markers used to isolate microvesicles secreted from prostate epithelial cells. B7-H3 is a protein biomarker found in transformed cells and is used to identify microvesicles from cancer cells. CD9, CD63 and CD81 are tetraspanins or transmembrane proteins found on most epithelial cells and on microvesicles secreted from epithelial cells. These tetraspanins act as general vesicle markers (see **Table 3).** Phycoerythrin (PE) labeled anti-tetraspanin antibodies are used for the detection of the various bound microvesicles in the assay. Depending on the level of binding of these antibodies to the microvesicles from a patient's plasma, a determination of a correlation with the presence or risk of prostate cancer is made.

[00884] In a retrospective study comparing prostate cancer patients with men who did not have prostate cancer this assay format showed an 83% sensitivity and 86% specificity. This biosignature will be tested with a set of prospectively gathered samples from all men scheduled for a prostate biopsy due to an elevated risk for prostate cancer.

[00885] In addition to the use of protein biomarkers, miRNA molecules will be added to the assay to improve the sensitivity and specificity of detecting and differentiating prostate cancer. This will be accomplished by collecting and concentrating the cMVs from plasma and extracting and measuring specific miRNA species and determining their correlation with prostate cancer. For example, miRNAs that have a high correlation with the presence of prostate cancer or metastatic prostate cancer are presented in the Examples above.

PREOMICS EXHIBIT 1002
Page 1175 of 1861

[00886] Objective: The objective of this prospective study is to identify and/or confirm a microvesicle-based prostate cancer specific biosignature that provides a high degree of accuracy in the detection of prostate cancer from a blood sample. More specifically the objective is to develop an assay for which will be an aid in the detection of prostate cancer in a population of men referred to a urologist for assessment of possible prostate cancer. Assay performance targets include ≥ 80% sensitivity and ≥ 80% specificity with an AUC of ≥ 0.80 on an ROC analysis based on the imperfect gold standard of a ≥10 core ultrasound-guided biopsy.

[00887] Intended Use: The circulating microvesic le (cMV) assay described in this Example is intended for the measurement of specified biomarkers in microvesicles in human plasma. The assay is intended to be used as an aid in the detection of prostate cancer in men aged 40 - 79 years considered to be at elevated risk for prostate cancer with an elevated PSA and/or abnormal digital rectal examination (DRE) and may be candidates for prostate biopsy.

[00888] Study Design: Blood samples will be collected from all men scheduled for a prostate biopsy who meet the stated inclusion criteria. Samples will be accessioned and stored in an appropriate format. Associated data collected on the Case Report Forms will be stored electronically, and paper copies will be filed accordingly. Blood will be processed into plasma as specified in the sample collection protocol (see **FIG. 25D),** frozen and shipped from collection locations (e.g., hospital or caregiver office) to the assay location where it will be processed and examined for both protein and nucleic acid biomarkers. Circulating microvesicles will be isolated and concentrated from plasma by a differential filtration procedure. Concentrated microvesicles will be examined for protein and miRNA biomarkers on a training set of at least 50 prostate cancer samples from men with biopsy confirmed prostate cancer as well as at least 50 samples from men with a negative biopsy from the same location. Samples from at least 3different collection locations across the United States will be used.

[00889] Both protein and RNAs will be examined for potential biomarkers correlated with the presence of PCa.

[00890] Methods: Protein biomarker selection will be performed on a Luminex 200 instrument system. Selected antibodies are conjugated to differentially addressable microspheres from Luminex Corp. according to the manufacturer's recommended protocol. After conjugation, the coated microspheres are washed, blocked by incubation in Starting Block Blocking Buffer in PBS (Thermo Scientific, cat # 37538), washed in phosphate buffered saline (PBS) and incubated with the concentrated cMVs from plasma as described below. Following capture of cMVs using bead bound anti-CD9, anti-B7H3, anti- PCSA and anti-PSMA, the microsphere-cMV complexes are washed and then incubated with phycoerythrin labeled detector antibodies (PE-CD9, PE-CD63 and PE-CD81) and washed prior to being read on the Luminex 200. The standard protocol comprises measuring the fluorescent signal from 100 microspheres and calculating the median fluorescent intensity (MFI) for each differentially addressable microsphere, each corresponding to a different capture antibody. Various combinations of detector and capture antibodies will be examined in addition to the tetraspanin detectors described above. For example, prostate or other marker biomarkers in **Table 5** will be assessed as desired.

[00891] Flow cytometry will be used to assay the total number of cMV in the various populations that are being assessed. Appropriate amounts of plasma samples will be diluted 100 times in PBS and then incubated for 15 min at room temperature in BD Trucount tubes (BD Biosciences, San Jose, CA) for quantification of events per sample. Trucount tubes contain an exactly number of fluorescent beads that can be compared with events for each sample by flow cytometry. Sample acquisition by FACS Canto II cytometer (BD Biosciences) and later analysis by FlowJo software (Tree Star, Inc., Ashland, OR) will reveal the number of sample events and number

PREOMICS EXHIBIT 1002
Page 1176 of 1861

of Trucount beads per tube. Finally, calculation of absolute number per sample will be obtained following instructions from BD and then adjusted by dilution factor.

[00892] MicroRNAs will also be examined from the cMVs from plasma samples. cMVs are concentrated and the miRNAs are extracted from the cMVs using a Trizol method. Briefly, cMVs are Rnase A (Epicentre Biotechnolgies, Madison, WI) treated (20 μg/mΐ for 20 min @ 37°C) followed by Trizol treatment (750 μΐ of Trizol LS to each 100 μΐ) and vortexed for 30 min. at 1400 rpm at room temperature. After centrifugation, the supernatant is collected and RNA is further purified with the miRNeasy 96 (Qiagen Inc., Valencia, CA) purification kit and stored at -80°C. 40 ng of RNA are reverse transcibed and run on the Exiqon qRT-PCR Human panel I and II (Exiqon, Inc, Woburn, MA) on an ABI 7900 (Applied Biosystems, Life Technologies, Carlsbad, CA). CT values are calculated by SDS 2.4 software (Applied Biosystems). All samples are normalized to inter plate calibrator and RT-PCR control.

[00893] Messenger RNA will also be examined from the cMVs from plasma samples. Messenger RNAs will be examined from the cMVs of plasma samples. First, cMVs will be isolated and treated with RNase A at 229 μg/mΐ for 20 minutes at 37C. Then the messenger RNA will be extracted using theTrizol method and purified with a Qiagen RNeasy mini kit precipitating with 70% ethanol. Sample RNA will be reverse transcribed and cy-3 labeled using Agilent's "Low Input Quick Amp Labeling" kit for one-color gene expression analysis according to the manufacturer's instructions. Labeled samples will be hybridized to Agilent's Whole Genome 44K v2 arrays and washed according to manufacturer's specifications (Agilent Technologies, Inc., Santa Clara, CA). Arrays will be scanned on an Agilent B scanner and data will be extracted with Feature Extractor (Agilent Technologies) software. Extracted data will be normalized with a global normalization method and analyzed with GeneSpring GX (Agilent Technologies) software.

[00894] Both miRNA and messenger RNA will be examined from specific subpopulations of cMVs from the plasma. For example, cMVs are concentrated then the population that is positive for PCSA is isolated using magnetic immunoprecipitation. Following isolation the miRNAs are extracted using a modified Trizol method. Briefly, cMVs are Rnase A (Epicentre) treated (20 μg/mΐ for 20 min @ 37°C) followed by Trizol treatment (750 ul of Trizol LS to each 100 μΐ) and vortexed for 30 min. @ 1400 rpm at room temperature. After centrifugation, the supernatant is collected and RNA is further purified with the miRNeasy 96 (Qiagen) purification kit and stored at -80°C. 40 ng of RNA are reverse transcibed and run on the Exiqon qRT-PCR Human panel I and II on an ABI 7900. CT values are calculated by SDS 2.4 software (Applied Biosystems). All samples are normalized to inter plate calibrator and RT-PCR control, and/or the number of cMVs. Messenger RNAs will be examined from the cMVs of plasma samples. First, cMVs will be isolated and treated with RNase A (Epicentre) at 229 μg/mΐ for 20 minutes at 37C. Then the messenger RNA will be extracted using a modified Trizol method and purified with a Qiagen RNeasy mini kit precipitating with 70% ethanol. Sample RNA will be reverse transcribed and cy-3 labeled using Agilent's "Low Input Quick Amp Labeling" kit for one-color gene expression analysis according to the manufacturer's instructions. Labeled samples will be hybridized to Agilent's Whole Genome 44K v2 arrays and washed according to manufacturer's specifications. Arrays will be scanned on an Agilent B scanner and data will be extracted with Feature Extractor (Agilent Technologies) software. Extracted data will be normalized with a global normalization method and analyzed with GeneSpring GX (Agilent Technologies) software.

PREOMICS EXHIBIT 1002
Page 1177 of 1861

[00895] Normalized analyte values will be imported into R (cran.org) and SAS software (SAS Institute Inc., Cary NC), subjected to quality control analysis and transformed prior to analysis. Analysis will proceed as follows:

1) Signature performance evaluation (for pre-specified or novel signatures)
    a. This sample set may be used to evaluate the performance of a signature that is fully specified prior to either the unblinding of clinical outcome *or* laboratory testing of samples. In such a case, the signature is considered *pre-specified* and must be applied, unmodified, to new analyte data on this sample set to obtain predicted outcomes for all samples. Performance of the pre-specified signature is evaluated by comparing predicted and true outcome (for example, in terms of diagnostic sensitivity, specificity, and accuracy). Statistics include performance estimates and confidence intervals.
    b. For signatures that are not pre-specified (i.e. that are derived with foreknowledge of *both* clinical outcome and laboratory testing results of samples), these samples may still be used to evaluate the performance of the signature. To ensure relatively unbiased estimates of performance, statistical analyses will be performed nested within a k-fold cross validation loop that will include *all* marker selection and class prediction steps, as described below.
2) Marker selection for novel signatures
    a. Markers are included in novel signatures only if they are shown to be statistically informative by testing for their association with disease outcome using a subset of commonly applied techniques, e.g.
        i. Welch test - robust parametric statistical test for difference between group means when variances are unequal.
        ii. Wilcoxon signed-rank test - robust non-parametric statistical test that can be interpreted as showing an improvement in ROC AUC (above 0.50)
        iii. Youden's J - calculated as the maximum combined sensitivity and specificity for a marker, across all possible diagnostic thresholds. Statistical significance is evaluated via permutation tests.
    b. Markers will only be judged statistically informative if the test is significant in the context of the number statistical tests performed. More specifically, we will adjust comparison-wise p-values for multiple testing - e.g. using false discovery rate thresholds or by control of family-wise error rates.
3) Formation of novel signatures: Assuming a subset of informative markers is identified in the preceding (marker selection) stage, novel multi-marker models are formed using well-established modeling techniques. Parameters for signatures will be estimated by training the models on the full training data set, and performance for the signature will be evaluated as described under "Signature performance evaluation" using the approach "for signatures that are not prespecified." We will focus on simple and well-established modeling techniques including: discriminant analysis, support vector machines, logistic regression, and decision trees. Results for all models will be reported.

[00896] Additional *aposteriori* analyses may be performed on the data set for clinical variables of interest- for example, number of previous biopsies, indication for biopsy and biopsy result (e.g. high-grade prostatic

PREOMICS EXHIBIT 1002
Page 1178 of 1861

intraepithelial neoplasia (HGPIN), atypical small acinar proliferation (ASAP), ATYPIA, benign prostatic hyperplasia (BPH) and prostatitis). Such analyses will be performed by introducing covariates or stratification variables into previously defined models. *A posteriori* tests will be performed only after assessment of data sufficiency, all tests and results will be recorded, and P-values will be corrected for multiple testing.

[00897] Patient Eligibility:

Inclusion Criteria

1. Gender: Male
2. Age range 40 to 79 years
3. Any Race or Ethnicity
4. Men scheduled for a prostate biopsy as part of their routine care and who agree to have their blood drawn within 7 days prior to the scheduled biopsy.
5. Prostate biopsy pathology report available to submit to Caris with all study forms and patient samples.
6. Level of comprehension to understand study and competence to sign consent.

Exclusion Criteria

1. Any prior or ongoing treatment for prostate cancer including prostatectomy or hormone therapy.
2. Previous diagnosis of cancer with the exception of prostate cancer or non-melanoma skin cancer.
3. Prostate biopsy within 30 days of blood collection.
4. DRE performed on day of blood collection before the blood draw was performed.
5. Decline phlebotomy.
6. Refusal to sign consent form.

[00898] The racial, gender (study limited to males) and ethnic characteristics of the individuals eligible for participation in the Prostate Blood Collection Project shall reflect the demographics of subjects receiving or seeking urological medical care. Subjects are included in accordance with these demographics. No individuals shall be excluded from participation in the Prostate Blood Collection Project based on race, national origin, ethnicity, disability or HIV status.

[00899] Patient or Data Selection Requirements: Data selection will be based on patient eligibility criteria and limited to the subset of data records corresponding to subjects included in this study (i.e. where laboratory analyte data is available). Data fields include those used for (non-PHI) donor identification, QC, and clinical interpretation as in **Table 30:**

Table 30: Patient Data

Field description	Comments
PHI de-identified patient identifier	Current biorepository donor barcode is sufficient
Site code	(May be included in the above de-identified patient barcode)
Race	White, Black or African American, American Indian or Alaska Native, Native Hawaiian or Pacific Islander, Asian, Unknown
Ethnicity	Hispanic or Latino, Not Hispanic or Latino, Unknown
Sex	MUST BE MALE
Age	In years (not date of birth)
Degree of inflammation	Acute, Chronic, Mixed, NA (not applicable), UNK (unknown)
DRE Result	Normal; Abnormal, unilateral < 50%; Abnormal, unilateral > 50%; Abnormal, bilateral; NA
Total PSA	ng/ml
Free PSA	%
Prostate Vol	Cc
PCA 3 Score	*Numeric*; Unk; NA
Reason for ordering index biopsy	Abnormal % Free PSA, Abnormal DRE finding, Abnormal Imaging data, Abnormal PSA density, Abnormal PSA velocity, Active surveillance, ASAP, Elevated PSA,

PREOMICS EXHIBIT 1002
Page 1179 of 1861

	Family history PCa, HGPIN, High PCA3, Rising PCA, Atypia
Number of previous prostate biopsies	
Were any previous prostate biopsies positive	
Degree of inflammation (associated with biopsy)	Acute, Chronic, mixed, NA, Unk
Biopsy cores	
Positive biopsy cores	
Primary Gleason	
Secondary Gleason	
Total Gleason	
AJCC Stage	TNM, Group Stage
Pathological diagnosis	Cancer, not cancer
Month/Year of diagnosis	(Not date of diagnosis, for HIPAA reasons)
Number of samples sent	Total samples sent, by tube type
HGPIN result	HGPIN or negative for HGPIN
ASAP result	ASAP or negative for ASAP
Atypia result	Atypia or negative for Atypia
Inflammation result	Chronic, Acute, or Mixed
Data entered by	Code indicating who entered data
Data QC'd by	Code indicating who performed QC on entered data
Status	Data partially entered, completely entered, QC'd, or similar

[00900] Patient or Data Collection Requirements: The data fields will be captured on the clinical data form at the facility where the blood is being drawn. Site personnel will use medical records and patient derived information to capture all data elements. Data collected for each case will be entered into the database within 48 hours of receipt. All data fields are required to be completed; if any are not, a query is sent to the site and a response is requested in 10 business days. Pre-selected data fields must be completed or the patient will be excluded from analysis.

[00901] One of skill will appreciate that a similar protocol can be followed to develop and confirm biosignatures comprising circulating biomarker in other settings, e.g., diagnostic, prognostic and/or theranostic assessment for other diseases and cancers than prostate cancer. For example, the biomarkers in **Table 5** can be used as part of a biosignature for other settings.

Example 44: Data Mining to Identify Biomarkers

[00902] MicroRNAs are known to regulate the expression of mRNA. An expression database has been created that contains information about the mRNA expression of many tumor types. The database contains data obtained using the Illumina DASL microarray (Illumina, Inc., San Diego, CA) for many thousands of patients. Circulating microvesicles (cMVs) contain microRNA as the dominant RNA species and also contain mRNAs. In this Example, an association was made between mRNA differentially expressed in cancer tumors from the expression database and those expressed in cMVs. The mRNAs found differentially expressed in tumor tissue were also used to find microRNA targets in cMVs.

[00903] Gene expression data from the expression database was evaluated to find the most statistically significant differentially expressed genes between prostate (PCa+), breast (BrCa), lung (LCa) and colorectal cancers (CRC) and matched normal tissue (PCa-), as well as between the cancer types **(Table 31).** Expression data (versions HT-12 and REF-8) for cancer samples (prostate, colorectal, breast, and lung) were analyzed to

PREOMICS EXHIBIT 1002
Page 1180 of 1861

detect genes differentially expressed between cancer types. Similarly, prostate cancer (PCa) samples were compared against prostate normal samples to detect prostate cancer specific probes. To perform the analysis, expression data were normalized prior to analysis by adopting a subset of 20 arbitrarily selected arrays (6 breast cancer, 5 colorectal cancer, 5 lung cancer, and 4 prostate cancer) to generate a quantile reference distribution. All arrays in the data set were then normalized against the reference distribution to ensure that each array shared the same quantile distribution. Next, normalized expression data were analyzed for each probe in the data set. Differentially expressed probes (and their corresponding genes) were detected by comparing each pair of classes (e.g. prostate cancer vs. breast cancer, and prostate cancer vs. prostate normal) using a F-score (a.k.a. Fisher's score) statistic. This statistic, which measures between vs. within class variation, was obtained by calculating the square of the mean group difference over the square of the sum of the group standard deviations. F-scores were set negative where the mean for the PCa+ samples was the lower of the two groups. Lastly, F-scores were sorted into descending sequence using the absolute value of the F-score, and the top up/down regulated markers were chosen from the list.

Table 31: Most Statistically Significant Differentially Expressed Genes Between PCa-i- Samples and Indicated Samples

Rank	PCa-	BrCa	CRC	LCa
1	*SEMG1*	KLK2	KLK2	KLK2
2	*MAP4K1*	KLK2	KLK2	KLK2
3	*CXCL13*	MAOA	KLK4	LRRC26
4	*GNAO1*	KLK4	LRRC26	LOC389816
5	*DST*	PVRL3	*CDX1*	KLK4
6	*AQP2*	SLC45A3	EEF1A2	CAB39L
7	*NELL2*	NLGN4Y	*FOXA2*	SPDEF
8	*TNNT3*	STX19	SPDEF	SIM2
9	*PRSS21*	CYorf14	*BAIAP2L2*	SLC45A3
10	*SNAI2*	C22orf32	FAM110B	PNPLA7
11	*BMP5*	PNPLA7	MIPOL1	TRIM36
12	*PGF*	SIM2	*CEACAM6*	*GSTP1*
13	*POU3F1*	FEV	SLC45A3	TRPV6
14	*ERCC1*	TRPM8	ADRB2	ASTN2
15	*TAF1C*	ARG2	LOC389816	*MUC1*
16	*KLHL5*	TRIM36	*C19orf33*	*MUC1*
17	*C16orf86*	ADRB2	ZNF613	ZNF613
18	*SMARCD3*	LRRC26	TRIM36	FAM110B
19	*PENK*	EIF1AY	*ERN2*	FEV
20	*SCML1*	SLC30A4	*TRIM31*	*CRIP1*

PCA+ Lower
PCA+ Higher

[00904] For prostate cancer, a list of the most significantly over and under-expressed genes was generated. These genes were compared to a list of mRNA that had been detected in cMVs from prostate cancer patients via microarray. One gene from the tissue list, AQP2, was also found to be expressed in cMVs. The list of up- and down-regulated genes from prostate tumor tissue was then mined using the TargetScan public database for microRNA that may influence the expression of these mRNAs. Matching microRNA was found for 11 of the 20 mRNA examined **(Table 32).** This list of microRNAs was then compared to a list of microRNAs that we found to be reliably detected in cMVs. This comparison revealed that 10 of the microRNAs that regulate the mRNA of interest in the prostate tumor tissue are also found in cMVs **(Table 32).**

PREOMICS EXHIBIT 1002
Page 1181 of 1861

Table 32: microRNA associated with differentially expressed mRNAs

PCa Up	TargetScan result	Detected in cMV?	PCa Down	TargetScan result	Detected in cMV?
ADCYAP1R1	no target	n/a	SEMG1	no target	n/a
HECTD3	miRs-26a+b	yes	MAP4K1	miR-342-5p	no
SLC44A4	no target	n/a	CXCL13	miR-186	yes
FASN	miRs-15/16/195/497/424	yes	GNAO1	miR-1271	no
MPG	no target	n/a	DST	miR-600	no
MIR720	no target	n/a	AQP2	miR-216b	no
PTBP1	miR-206	yes	NELL2	miR-519 family	no
CPSF1	no target	n/a	TNNT3	no target	n/a
C2orf56	no target	n/a	PRSS21	miR-206	yes
HCRTR1	no target	n/a	SNAI2	miR-203	yes

[00905] Additionally, mRNAs that are found to be differentially expressed are often indicative of differences in the protein level. The results of this mining activity have identified proteins (e.g., KLK2) associated with cMVs that can be used to differentiate prostate cancer from other cancers, including breast, lung, and colon cancer. KLK2 is known to be associated with prostatic tissue.

Example 45: microRNA functional assay

[00906] MicroRNAs can be found circulating in the blood encapsulated in microvesicles, HDL and LDL particles as well as components of ribonucleoprotein complexes (RNPs). microRNA can be detected using available technologies such as RT-qPCR or next generation sequencing. However, microRNA that in a biologically active state are bound and activated by one of the Argonaute proteins (Agol-4). This Example presents an assay that can detect functional activity of a given microRNA within a sample from various sources (including without limitation cell lysates, bodily fluids, plasma, serum, isolated microvesicles, etc) in a single reaction.

[00907] The assay comprises microbeads, a biotin conjugated synthetic RNA molecule, streptavidin-PE, recombinant Argonaute 2 and RISC (RNA-Induced Silencing Complex) reaction buffer components. Components of the assay are shown in **FIG. 26.** As shown in **FIG. 26A,** the biotin conjugated synthetic RNA molecule contains a 3' linker/extender region **262,** a central miRNA targeting region **263** and a second 5'linker/extension region **264.** The RNA is attached to a microbead **261** on the 3'end and the 5'end is conjugated with biotin **266.** The central miRNA targeting region **263** is designed to complement a miRNA sequence of interest. Any microRNA of interest can be used in the assay; for the sake of example only let-7a is used here. Streptavidin-PE (Phycoerythrin) **265** is used to label the biotin end of the RNA. If target let-7a is present in the sample and is bound/associated with an Ago protein **267,** e.g., recombinant Ago2 (rAgo2), let-7a will bind the complementary microRNA targeting region **263** and subsequently cleave the synthetic RNA at region **263** through the endonucleolytic cleavage activity of Argonaute 2. *See* step **268** in **FIG. 26.** Once cleaved, the 5' end of the synthetic RNA molecule is released, thereby separating the biotin/Streptavidin-PE complex from the microbead **261.** See **FIG. 26B.** Next, the microbeads are isolated and washed to remove the cleaved RNA, thereby leaving only the remaining uncleaved material as well as any cleaved RNA. After this wash step, the difference in PE signal correlates with the concentration and activity of the Ago-bound target microRNA **267** present in the original assay. In this Example, the quantity of Ago-bound let-7a in the input

PREOMICS EXHIBIT 1002
Page 1182 of 1861

sample determines the level of RNA cleaved. For example, if let-7a is not present, the synthetic RNA target region **263** will remain uncleaved and the signal strength will be unchanged.

[00908] **FIGs. 26C-E** illustrate schematically various sources of RNA that can be used as input for the assay. **FIG. 26C** illustrates microRNA **268** bound to an Ago protein **269** to form a ribonucleic acid complex **267. FIG. 26D** illustrates immunoprecipitation of an Argonaute - microRNA complex **267** using a binding agent to Ago **2610. FIG. 26E** illustrates direct analysis of Argonaute - microRNA complex **267,** e.g., from a cell lysate, bodily fluid, or lysed microvesicle.

Example 46: Circulating Microvesicles (cMVs) in Prostate Cancer Patient Samples

[00909] In this Example, cMVs are profiled in prostate cancer and related diseases. Methodology is similar to **Examples 20-24.** Generally, capture agents (antibodies and/or aptamers) are tethered to fluorescently labeled microbeads and incubated with cMVs from patient plasma. The captured cMVs are detected with fluorescently labeled detector agents (antibodies and/or aptamers). Fluorescent signals are then used to compare levels of specific cMV populations in the patient samples. A total of 129 intended use samples are included in the study, including 61 cancers and 68 non-cancers. Patient characteristics are shown in **Tables 33-36:**

Table 33: Biopsy Result

Patient Biopsy Result	Number
Benign/inflammation	49
HGPIN/Atypia	19
Cancer	61

Table 34: Pathology of "Normal" Samples

"Normal" Pathology	Number
Benign	32
Inflammation	17
Atypia/ASAP	1
HGPIN	18

Table 35: Patient Clinical Data

Demographic	Not Cancer	Cancers
Avg. PSA	5.0	6.2
Avg. Age	61.6	65.3
Number	68	61

Table 36: Patient Race

Race	Normal	Cancer	Total
Caucasian	53	49	102
Black or African American	5	3	8
Asian	1	2	3
Native Hawaiian or other Pacific Islander	0	1	1
American Indian or Alaska Native	1	0	1

[00910] Capture and detector antibodies are shown in **Table 37:**

PREOMICS EXHIBIT 1002
Page 1183 of 1861

Table 37: Capture and Detector Antibodies

Antibody	Target Abbreviation	Clone	Vendor	Catalog Number
Anti filamin A alpha antibody	FLNA	4E10-1B2	Sigma-Aldrich	WH0002316M1
Anti Interleukin 8 antibody	IL8	790128G2	Thermo scientific pierce	OMA1-03346
Anti Human Epidermal growth factor Receptor 3 antibody	HER 3 (ErbB3)	Polyclonal	US Biological	E3451-36A
Anti-cluster of differentiation 9 antibody	CD9	209306	R&D Systems	MAB1880
Anti macrophage migration inhibihitory factor antibody	MIF	2Ar3	Genetex	GTX14575
Anti heat shock protein antibody	HSP70	W27	Biolegend	648002
Anti insulin induced gene 2 antibody	INSIG-2	H40	Santa Cruz	sc-66936
Anti brain derived neurotrophic factor antibody	BDNF	polyclonal	US Biological	B2700-02D
Anti Mnllerian inhibiting substance receptor II antibody	MIS RII	polyclonal	R&D	AF4749
Anti epidermal growth factor antibody	EGFR	af231	BD biosciences	555996
Anti Interleukin-1B antibody	IL-1B	2A8	Sigma Aldrich	WH0003553M1
Anti human inactive complement component 3b antibody	iC3b	013III-1.16	Thermo	MA1-82814
Anti Prostate specific membrane antibody	PSMA	LNI-17	Biolegend	342502
Anti chicken IgY antibody	ChickenIgY	polyclonal	Abcam	ab50579
Anti alpha fetal protein antibody	AFP	Monoclonal	Abcam	ab54745
Anti prostate cell surface antibody	PCSA	5 E 10	Inhouse	MAB4089
Anti cluster of differentiation 63 antibody	CD63	H5C6	BD pharmingen	556019
Anti B-cell novel protein1 antibody	BCNP	polyclonal	abcam	ab59781
Anti Mucin 1, cell surface associated protein antibody	MUC1	Vu4H5	Santa Cruz	sc7313
Anti Abelson murine leukemia viral oncogene homolog 2 antibody	ABL2	6D5	ABDserotec	MCA2898Z
Anti cluster of differentiation 81 antibody	CD81	JS-81	BD pharmingen	555675
Anti Cluster of differentiation 276 antibody	B7H3	MIH35	BioLegend	135602
Anti S100 calcium binding protein A4 antibody	S100-A4	1f12-1g7	Sigma aldrich	WH0006275M1
Anti Cytidine deaminase antibody	CDA	Polyclonal	abcam	ab35251
Anti kallikrein-related peptidase 2 antibody	KLK2	3C5	Novus Biologicals	H00003817-M03
Anti Prostate specific antibody	PSA	B731M	My bioscource	MBS312739
Anti Cluster of differentiation 46 antibody	CD46	344519	R&D systems	MAB2005
Anti Neurokinin-A antibody	NK-2R(C-21)	Polyclonal	Santacruz	sc-14121
Anti wingless type integration site family antibody	wnt-5a(C-16)	Polyclonal	Santacruz	sc-23698
Anti Cluster of differntiation 24 antibody (Heat Stable antigen)	CD24	ml5	BD biosciences	bd 555426
Anti Tissue inhibitor of metallo proteinase-1 antibody	TIMP-1	4D12	Sigma-Aldrich	WH0007076M1
Anti dead box protein 1 antibody	DDX-1	22	BD Transduction laboratories	D84920
Anti proviral integration site antibody	PIM1	1C10	Novus Biologicals	H00005292-M08
Anti Regenerating islet-derived family, member 4 antibody	Reg IV	MM0254-9B21	Abcam	ab89917
Anti Matrixmetallo Proteinase 9 antibody	MMP9	SB15C	Novus biologicals	NBP1-28617
Anti Ephrin-A receptor 2 antibody	EphA2	ka5h5	Santa Cruz	sc101377
Anti Tumor Microenvironment of Metastasis 211 antibody	TMEM211	c15	Santa Cruz	sc86534
Anti Histone-lysine N-methyltransferase antibody	EZH2	2C3	Sigma aldrich	WH0002146M1
Anti Prostate specific antibody	PSA	BGN/PSA6	Novus	NB100-66506

PREOMICS EXHIBIT 1002
Page 1184 of 1861

			Biologicals	
Anti Delta like protein 4 antibody	DLL4	207822	R&D systems	MAB1389
Anti Tumor necrosis factor like weak inducer of apoptosis	TWEAK	Poly	US biological	T9185-01
Anti Apoptotic linked gene product 2 Interacting Protein X antibody	ALIX	3A9	Thermo scientific pierce	MA1-83977
Anti human trophoblast cell-surface antibody	Trop2	yy01	Santa Cruz	sc80406
Anti human Fas Ligand antibody	FASL	9i01	US Biologicals	F0019-66V
Anti unc 93 homolog A antibody	UNC93A	I13	Santa Cruz	sc135541
Anti glyco protein a33 antibody	A33	g20	Santa Cruz	sc33014
Anti Aurora Bkinase (serine/threonine-protein kinase 6) antibody	AURKB	6A6	Novus Biologicals	H00009212-M01A
Anti c-erb2 antibody	C-erbB2	42/c-erbB2-2	BD Biosciences	610161
Anti Cluster of differntiation 10 antibody (Heat Stable antigen)	CD10	HI10a	BD Pharmingen	555373
Anti Secreted protein acidic antibody rich in cysteine antibody	SPARC	122511	R&D systems	MAB941
Anti ferritin f31 antibody	FRT	F31	Santa Cruz	sc-51888
Anti chemokine (C-X-C motif) receptor 31 antibody	CXCR3	49801	R&D systems	MAB160
Anti cytokeratin 19 fragment antibody	CYFRA21-1	1603	MedixMab	102221
Anti carcino embryogenic antibody	CD66e CEA	Polyclonal	US Biological	C1300-08
Anti interleukin 7receptor antibody	IL7 R alpha/CD127	40131	R&D systems	MAB306
Anti Six Transmembrane Epithelial Antigen of the Prostate 1 antibody	STEAP	polyclonal	Santacruz	sc-25514
Anti single minded protein 2 antibody	SIM2 (C-15)	Polyclonal	Santacruz	sc-8715
Anti Mucin 17, cell surface associated protein antibody	MUC17	c19	Santa Cruz	sc32602
Anti Vascular Endothelial Growth Factor Receptor 2 antibody	hVEGFR2	89106	R&D systems	MAB3572
Anti Mucin 2, cell surface associated protein antibody	MUC2	H-300	Santa Cruz	sc15334
Anti disintegrin and metalloproteinase domain 10 antibody	ADAM10	163003	R&D systems	MAB1427
Anti Aspartyl/asparaginyl β-hydroxylase(A10) antibody	ASPH (A-10)	A-10	Santa Cruz	sc-271391
Anti carbohydrate antigen 125 antibody	CA125 (MUC16)	8J453	US Biological	C0050-01D
Anti GATA binding protein 2 antibody	GATA2	2D11	Sigma-Aldrich	WH0002624M1
Anti Receptor for advanced glycosylation end products antibody	RAGE	polyclonal	Abcam	ab30381
Anti surfactant protein-C antibody	SPC	Polyclonal	US Biological	U2575-03
Anti Trefoil factor 3 (intestinal) antibody	TFF3	3D9	Sigma-Aldrich	WH0007033M1
Anti tyrosine Kinase B antibody	TrKB (poly)	Polyclonal	Novus biologicals	NB100-92063
Anti-cluster of differentiation 9 antibody	CD9	209306	R&D Systems	MAB1880
Anti Apolipoprotein J Antibody	Apo J/CLU	2F12	NovusBiologicals	H00001191-M02
Anti carbohydrate 19-9 antibody	CA-19-9	3H606	US Biological	C0075-13B
Anti cytidine and dCMP deaminase domain containing 1 antibody	CDADC1	1A2	Sigma-Aldrich	WH0081602M1
Anti Galactose metabolism regulator 3 antibody	GAL3	B2C10	Santa Cruz	sc-32790
Anti heat shock 27kDa protein 1 antibody	HSPB1	3G3	Sigma-Aldrich	WH0003315-M4
Anti receptor activator of NFκB antibody	RANK	80704	R&D systems	MAB683
Anti-Human granulocyte macrophage colony stimulating factor antibody	GM-CSF	BVD2 23B6	Invitrogen	AHC2 012
Anti-Secreted Phospho Protein 1 antibody	SPP1	IE10	Sigma	WH0006696M1
Anti chicken IgY antibody	ChickenIgY	polyclonal	Abcam	ab50579

PREOMICS EXHIBIT 1002
Page 1185 of 1861

Anti prostate cell surface antibody	PCSA	5 E 10	Inhouse	MAB4089
Anti cluster of differentiation 63 antibody	CD63 Novus	CLB-180	NovusBiologicals	NBP1-42306
Anti immune cosmitulatory protein antibody	B7H4	Polyclonal	US Biological	B0000-35A
Anti Transglutaminase-2 antibody	TGM2	2F4	Sigma Aldrich	WH0007052M10
Anti cluster of differentiation 81 antibody	CD81	JS-81	BD pharmingen	555675
Anti Cluster of differentiation 276 antibody	B7H3 R&D	185504	R&D systems	MAB1027
Anti Milk fat globule-EGF factor 8 protein antibody	MFG-E8	278918	R&D systems	MAB27671
Anti Laminin antibody	LAMN	2Q592	US Biological	L1225-20
Anti Macrophage colony-stimulating factor antibody	M-CSF	21113	R&D systems	MAB616
PSMA Aptamer seq 4	PSMA Aptamer	4	IDT	57926604
Anti Integrin α5 (A-11) antibody	Integrin	A-11	Santacruz	sc-166665
Anti Apo lipo protein antibody	ALPL	4H1	sigma aldrich	WH0000249M1
Anti c-reactive protein antibody	CRP	CRP8	Abcam	ab13426
Anti Vascular endothelial growth factor A antibody	VEGF A	5J63	US Biological	V2110-05D
Anti human interleukin 6 unconjugated antibody	IL6 Unc	8H12	Invitrogen	AHC0762
Anti Prostatic binding protein antibody	PBP	2G2-1F1	Novus Biologicals	H00005037-M01
Anti Cluster of differentiation 59 (MEM-43) antibody	CD59(MEM-43)	MEM-43	gentex	GTX74620
Anti TNF-related apoptosis-inducing ligand receptor 4 antibody	Trail-R4	104918	R&D systems	MAB633
Anti Six Transmembrane Epithelial Antigen of the Prostate 4 antibody	STEAP4	8H178	US Biological	S7500-02
Anti prolactin Monoclonal antibody	PRL	6F11	Thermo Scientific Pierce	MA1-10597
Anti Matrix metallo Proteinase 7 antibody	MMP7	polyclonal	Novus biologicals	NB300-1000
Muc1 Aptamer Seq 2A	Muc1 Aptamer	2A	IDT	55403580
Anti Cluster of differntiation 44 antibody	CD44	5C10	Novus Biologicals	NBP1-04276
Anti runt-related transcription factor 2 antibody	RUNX2	1D8	Sigma aldrich	WH0000860M1
Anti serpin peptidase inhibitor, clade B member 3 antibody	SERPINB3	2F5	Sigma aldrich	WH0006317M1
Anti mammaglobin A(C-16) antibody	Mammoglobin	polyclonal	Santa Cruz	sc-48328
Anti serum amyloid A antibody	ALA	291	abcam	ab18713
Anti Delta like protein 4 antibody	DLL4	Monoclonal	Abcam	ab61031
Anti cluster of differentiation 41 antibody	CD41	PM6/248	Mybiosource	MBS210248
Anti cluster of differentiation 151 antibody	CD151	210127	R&D systems	MAB1884
Anti SAM pointed domain containing ets transcription factor antibody	SPDEF	4A5	Novus Biologicals	H00025803-M01
Anti 5' nucleotidase antibody	NT5E (CD73)	606112	R&D systems	MAB5795
Anti seprase antibody	seprase/FAP	427819	R&D	MAB3715
Anti Neutrophil gelatinase-associated lipocalin antibody	NGAL	h130	Santa Cruz	sc50350
Anti Epithelial cellular adhesion molecule antibody	Epcam	158206	R&D systems	MAB 9601
Anti platelet-derived growth factor receptor,beta,subunit antibody	PDGFRB	PR7212	R&D systems	MAB1263
Anti saccharomyces cerevisiae antibody	ASCA	Polyclonal	abcam	ab19731
Anti tumor protein 53 antibody	p53	DO-7	BioLegend	645802
Anti Interleukin 6 receptor antibody	IL6 R	2G5	Sigma-Aldrich	WH0003570M1
Anti Flagellin antibody	C-Bir	polyclonal	abcam	ab93713
Anti Aspartyl/asparaginyl β-hydroxylase(DO1 P) antibody	ASPH (D01P)	Polyclonal	Novus	H00000444-D01P
Anti collapsin response mediator protein 2 antibody	CRMP-2	Polyclonal	AbD Serotec	AHP1255

PREOMICS EXHIBIT 1002
Page 1186 of 1861

Anti Ets related gene antibody	ERG	Polyclonal	sigma aldrich	SAB2500363
Anti-hNCAM/CD56 antibody	Ncam	301040	R&D	MAB2408
Anti Chemokine (C-X-C motif) ligand 12 antibody	CXCL12	79018	R&D systems	MAB350
Anti haptoglobin antibody	HAP	1.C.1	USBIO	HI820-05
Anti TNF-related apoptosis-inducing ligand receptor 2 antibody	Trail-R2	Polyclonal	Thermo scientific pierce	PA1-23497
Anti Human gro alpha antibody	Gro-alpha	Polyclonal	GeneTex	GTX10376
Anti tumor susceptibility gene 101 antibody	Tsg 101	Y16J	Santacruz	sc-101254
Anti NADH ubiquitinone 1 beta subcomplex subunit7 antibody	NDUFB7	polyclonal	Abgent	AP6658b

[00911] Five detector agents were used, comprising: 1) combination of tetraspanins CD9, CD63, CD81 ; 2) CD81 alone; 3) PCSA; 4) MUC2; and 5) MFG-E8. Combinations of detector agents along with microbead-tethered capture agents are shown in **Table 38.** In the table, the capture and/or detector agents comprised antibodies unless noted as aptamers. The first row identifies the Detector agents. Beneath each detector is the list of capture agents used with the detector. Chicken IgY was run as a control.

Table 38: Capture and Detector Agent Combinations

CD9, CD63, CD81	CD81	PCSA	MUC2	MFG-E8
FLNA	FLNA	FLNA	FLNA	FLNA
IL8	IL8	IL8	IL8	IL8
HER3(ErbB3)	HER3(ErbB3)	HER3(ErbB3)	HER3(ErbB3)	HER3(ErbB3)
CD9	CD9	CD9	CD9	CD9
MIF	MIF	MIF	MIF	MIF
HSP70	HSP70	HSP70	HSP70	HSP70
INSIG-2	INSIG-2	INSIG-2	INSIG-2	INSIG-2
BDNF	BDNF	BDNF	BDNF	BDNF
MISRII	MISRII	MISRII	MISRII	MISRII
EGFR	EGFR	EGFR	EGFR	EGFR
IL-1B	IL-1B	IL-1B	IL-1B	IL-1B
iC3b	iC3b	iC3b	iC3b	iC3b
PSMA	PSMA	PSMA	PSMA	PSMA
ChickenIgY	ChickenIgY	ChickenIgY	ChickenIgY	ChickenIgY
AFP	AFP	AFP	AFP	AFP
PCSA	PCSA	PCSA	PCSA	PCSA
CD63	CD63	CD63	CD63	CD63
BCNP	BCNP	BCNP	BCNP	BCNP
MUC1	MUC1	MUC1	MUC1	MUC1
ABL2	ABL2	ABL2	ABL2	ABL2
CD81	CD81	CD81	CD81	CD81
B7H3	B7H3	B7H3	B7H3	B7H3
S100-A4	S100-A4	S100-A4	S100-A4	S100-A4
CDA	CDA	CDA	CDA	CDA
KLK2	KLK2	KLK2	KLK2	KLK2
PSA	PSA	PSA	PSA	PSA
CD46	CD46	CD46	CD46	CD46
NK-2R(C-21)	NK-2R(C-21)	NK-2R(C-21)	NK-2R(C-21)	NK-2R(C-21)
wnt-5a(C-16)	wnt-5a(C-16)	wnt-5a(C-16)	wnt-5a(C-16)	wnt-5a(C-16)
CD24	CD24	CD24	CD24	CD24
TIMP-1	TIMP-1	TIMP-1	TIMP-1	TIMP-1
DDX-1	DDX-1	DDX-1	DDX-1	DDX-1
PIM1	PIM1	PIM1	PIM1	PIM1

PREOMICS EXHIBIT 1002
Page 1187 of 1861

ReglV	ReglV	ReglV	ReglV	ReglV
MMP9	MMP9	MMP9	MMP9	MMP9
EphA2	EphA2	EphA2	EphA2	EphA2
TMEM21 1	TMEM21 1	TMEM21 1	TMEM21 1	TMEM21 1
EZH2	EZH2	EZH2	EZH2	EZH2
PSA	PSA	PSA	PSA	PSA
DLL4	DLL4	DLL4	DLL4	DLL4
TWEAK	TWEAK	TWEAK	TWEAK	TWEAK
ALIX	ALEX	ALIX	ALIX	ALIX
Trop2	Trop2	Trop2	Trop2	Trop2
FASL	FASL	FASL	FASL	FASL
UNC93A	UNC93A	UNC93A	UNC93A	UNC93A
A33	A33	A33	A33	A33
AURKB	AURKB	AURKB	AURKB	AURKB
ErbB2	ErbB2	ErbB2	ErbB2	ErbB2
CD10	CD10	CD10	CD10	CD10
SPARC	SPARC	SPARC	SPARC	SPARC
FRT	FRT	FRT	FRT	FRT
CXCR3	CXCR3	CXCR3	CXCR3	CXCR3
CYFRA21-1	CYFRA21-1	CYFRA2 1-1	CYFRA21-1	CYFRA21-1
CD66eCEA	CD66eCEA	CD66eCEA	CD66eCEA	CD66eCEA
IL7Ralpha/CD127	IL7Ralpha/CD127	IL7Ralpha/CD127	IL7Ralpha/CD127	IL7Ralpha/CD127
STEAP	STEAP	STEAP	STEAP	STEAP
SIM2(C-15)	SIM2(C-15)	SIM2(C-15)	SIM2(C-15)	SIM2(C-15)
MUC17	MUC17	MUC17	MUC17	MUC17
hVEGFR2	WEGFR2	hVEGFR2	hVEGFR2	hVEGFR2
MUC2	MUC2	MUC2	MUC2	MUC2
ADAM 10	ADAM 10	ADAM 10	ADAM 10	ADAM 10
ASPH(A-IO)	ASPH(A-IO)	ASPH(A-IO)	ASPH(A-10)	ASPH(A-IO)
CA125(MUC16)	CA125(MUC16)	CA125(MUC16)	CA125(MUC16)	CA125(MUC16)
GATA2	GATA2	GATA2	GATA2	GATA2
RAGE	RAGE	RAGE	RAGE	RAGE
SPC	SPC	SPC	SPC	SPC
TFF3	TFF3	TFF3	TFF3	TFF3
TrKB(poly)	TrKB(poly)	TrKB(poly)	TrKB(poly)	TrKB(poly)
CD9	CD9	CD9	CD9	CD9
ApoJ/CLU	ApoJ/CLU	ApoJ/CLU	ApoJ/CLU	ApoJ/CLU
CA-19-9	CA-19-9	CA-19-9	CA-19-9	CA-19-9
CDADC1	CDADC1	CDADC1	CDADC 1	CDADC1
GAL3	GAL3	GAL3	GAL3	GAL3
HSPB1	HSPB1	HSPB1	HSPB1	HSPB1
RANK	RANK	RANK	RANK	RANK
GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF
SPP1	SPP1	SPP1	SPP1	SPP1
ChickenlgY	ChickenlgY	ChickenlgY	ChickenlgY	ChickenlgY
PCSA	PCSA	PCSA	PCSA	PCSA
CD63	CD63	CD63	CD63	CD63
B7H4	B7H4	B7H4	B7H4	B7H4
TGM2	TGM2	TGM2	TGM2	TGM2
CD81	CD81	CD81	CD81	CD81
B7H3	B7H3	B7H3	B7H3	B7H3
MFG-E8	MFG-E8	MFG-E8	MFG-E8	MFG-E8

PREOMICS EXHIBIT 1002
Page 1188 of 1861

LAMN	LAMN	LAMN	LAMN	LAMN
M-CSF	M-CSF	M-CSF	M-CSF	M-CSF
PSMAAptamer	PSMAAptamer	PSMAAptamer	PSMAAptamer	PSMAAptamer
Integrin	Integrin	Integrin	Integrin	Integrin
ALPL	ALPL	ALPL	ALPL	ALPL
CRP	CRP	CRP	CRP	CRP
VEGFA	VEGFA	VEGFA	VEGFA	VEGFA
IL6Unc	IL6Unc	IL6Unc	IL6Unc	IL6Unc
PBP	PBP	PBP	PBP	PBP
CD59(MEM-43)	CD59(MEM-43)	CD59(MEM-43)	CD59(MEM-43)	CD59(MEM-43)
Trail-R4	Trail-R4	Trail-R4	Trail-R4	Trail-R4
STEAP4	STEAP4	STEAP4	STEAP4	STEAP4
PRL	PRL	PRL	PRL	PRL
MMP7	MMP7	MMP7	MMP7	MMP7
Muc1Aptamer	Muc1Aptamer	Muc1Aptamer	Muc1Aptamer	Muc1Aptamer
CD44	CD44	CD44	CD44	CD44
RUNX2	RUNX2	RUNX2	RUNX2	RUNX2
SERPINB3	SERPINB3	SERPINB3	SERPINB3	SERPINB3
Mammoglobin	Mammoglobin	Mammoglobin	Mammoglobin	Mammoglobin
ALA	ALA	ALA	ALA	ALA
DLL4	DLL4	DLL4	DLL4	DLL4
CD41	CD41	CD41	CD41	CD41
CD151	CD151	CD151	CD151	CD151
SPDEF	SPDEF	SPDEF	SPDEF	SPDEF
NT5E (CD73)	NT5E (CD73)	NT5E (CD73)	NT5E (CD73)	NT5E (CD73)
seprase/FAP	seprase/FAP	seprase/FAP	seprase/FAP	seprase/FAP
NGAL	NGAL	NGAL	NGAL	NGAL
Epcam	Epcam	Epcam	Epcam	Epcam
PDGFRB	PDGFRB	PDGFRB	PDGFRB	PDGFRB
ASCA	ASCA	ASCA	ASCA	ASCA
p53	p53	p53	p53	p53
IL6R	IL6R	IL6R	IL6R	IL6R
C-Bir	C-Bir	C-Bir	C-Bir	C-Bir
ASPH(D01P)	ASPH(D01P)	ASPH(D01P)	ASPH(D01P)	ASPH(D01P)
CRMP-2	CRMP-2	CRMP-2	CRMP-2	CRMP-2
ERG	ERG	ERG	ERG	ERG
Ncam	Ncam	Ncam	Ncam	Ncam
CXCL12	CXCL12	CXCL12	CXCL12	CXCL12
HAP	HAP	HAP	HAP	HAP
Trail-R2	Trail-R2	Trail-R2	Trail-R2	Trail-R2
Gro-alpha	Gro-alpha	Gro-alpha	Gro-alpha	Gro-alpha
Tsg101	Tsg101	Tsg101	Tsg101	Tsg101
NDUFB7	NDUFB7	NDUFB7	NDUFB7	NDUFB7

[00912] The above panels were run on the microbead system and fluorescence intensities were compared for each combination of detector / capture agents **(Table 38)** between the prostate cancer and normal samples. The performance of PSA testing in the cohort is shown in **Table 39.** Thirty three marker combinations were identified that each individually perform significantly better than PSA in this cohort, as shown in **Table 40.** The results were reanalyzed using background corrected and normalized data against the detected Chicken IgY for each patient, as shown in **Table 41.**

PREOMICS EXHIBIT 1002
Page 1189 of 1861

Table 39: PSA Performance

PSA Cutoff (ng/ml)	Sensitivity	Specificity	Estimated Accuracy
2.5	93%	10%	52%
4	80%	36%	58%
10	11%	91%	51%

Table 40: Top Performing Combinations of Detectors and Capture Agents

Detector	Capture	Youden-J	p-value	False Discovery Rate	Estimated Accuracy
MFGE8	MUC17	0.37	< .001	0.09	68%
CD81	S100 A4	0.35	< .001	0.09	67%
Muc2	SPARC	0.33	< .001	0.09	67%
CD81	KLK2	0.34	< .001	0.09	67%
MFGE8	GAL3	0.39	< .001	0.09	70%
MFGE8	Tsg101	0.32	< .001	0.09	66%
Muc2	seprase /FAP	0.36	< .001	0.09	68%
CD81	ADAM10	0.36	< .001	0.09	68%
MFGE8	MUC1	0.34	< .001	0.09	67%
MFGE8	A33	0.33	< .001	0.10	67%
MFGE8	ASPH(A 10)	0.32	< .001	0.10	66%
Muc2	CD63	0.37	< .001	0.10	69%
Muc2	FASL	0.33	< .001	0.10	66%
MFGE8	ERG	0.34	< .001	0.10	67%
CD81	CD66eCEA	0.32	< .001	0.10	66%
MFGE8	HER3(ErbB3)	0.34	< .001	0.10	67%
MFGE8	NK 2R(C 21)	0.35	< .001	0.10	68%
MFGE8	TWEAK	0.32	< .001	0.10	66%
MFGE8	SIM2(C 15)	0.33	< .001	0.10	67%
CD81	B7H4	0.34	< .001	0.10	67%
MFGE8	Mammoglobin	0.33	< .001	0.10	67%
MFGE8	seprase /FAP	0.30	< .001	0.10	65%
MFGE8	IL8	0.32	< .001	0.10	66%
MFGE8	FRT	0.31	< .001	0.10	65%
Tets	UNC93A	0.35	< .001	0.10	67%
MFGE8	SPC	0.33	< .001	0.10	67%
MFGE8	TGM2	0.33	< .001	0.10	66%
MFGE8	HAP	0.30	< .001	0.10	65%
Muc2	Epcam	0.31	< .001	0.10	65%
Muc2	C Bir	0.33	< .001	0.10	67%
Muc2	Ncam	0.31	< .001	0.10	66%
MFGE8	MMP9	0.31	< .001	0.10	66%
MFGE8	EphA2	0.33	< .001	0.10	67%

Table 41: Top Performing Combinations of Detectors and Capture Agents (Alternate Normalization)

Detector	Capture	Wilcoxon P-value	Wilcoxon FDR	Estimated Accuracy
CD81	S100-A4	0.0083	0.26	64%
CD81	ADAM10	0.0035	0.169	64%
CD9/63/81	UNC93A	0.0030	0.166	63%
MFGE8	MUC1	0.002	0.149	66%
MFGE8	TGM2	0.02	0.34	66%

PREOMICS EXHIBIT 1002
Page 1190 of 1861

MFGE8	Integrin	0.0004	0.07	66%
MFGE8	NK-2R(C-21)	0.0004	0.07	66%
MFGE8	PBP	0.0035	0.17	66%
MFGE8	Trail-R4	0.002	0.15	65%
MFGE8	EphA2	0.0014	0.12	66%
MFGE8	CD9	0.013	0.29	63%
MFGE8	Mammoglobin	0.0004	0.07	67%
MFGE8	A33	0.002	0.15	66%
MFGE8	seprase/FAP	0.005	0.2	63%
MFGE8	FRT	0.004	0.19	64%
MFGE8	SIM2(C-15)	0.0005	0.07	67%
MFGE8	MUC17	0.0008	0.08	67%
MFGE8	GAL3	0.0008	0.08	66%
MFGE8	ASPH(A-10)	0.004	0.19	65%
MFGE8	CXCL12	0.005	0.19	64%

[00913] The data in **Tables 40** and **41** show several marker panels that individually outperform PSA to distinguish prostate cancer from non-cancer (see **Table 39).** The marker combinations in **Table 41** were next analyzed to determine how performance varies by patient pathological subtype. In this scenario, the inflammation subtype was differentiated from prostate cancer with poorer performance than other benign diseases or HGPIN, as shown in **Table 42.**

Table 42: Differentiating Prostate Cancer (PCa) From Other Patient Pathological Subtypes

		Percent of Samples Called Correctly				
Detector	**Capture**	**Benign**	**HGPIN**	**Inflammation**	**PCa**	**PCa–watchful waiting**
CD81	S100-A4	56.2	61.1	35.3	75.9	85.7
CD81	ADAM10	46.9	44.4	29.4	87	100
MFGE8	MUC1	71.9	72.2	52.9	63	71.4
MFGE8	TGM2	62.5	66.7	41.2	70.4	100
MFGE8	Integrin	68.8	61.1	52.9	68.5	71.4
MFGE8	NK-2R(C-21)	65.6	55.6	35.3	77.8	71.4
MFGE8	PBP	68.8	55.6	52.9	70.4	71.4
MFGE8	Trail-R4	65.6	72.2	52.9	68.5	42.9
MFGE8	EphA2	62.5	61.1	41.2	75.9	71.4
MFGE8	CD9	46.9	38.9	47.1	85.2	71.4
MFGE8	Mammoglobin	65.6	61.1	52.9	75.9	57.1
MFGE8	A33	65.6	72.2	47.1	70.4	71.4
MFGE8	seprase/FAP	53.1	50	35.3	77.8	100
MFGE8	FRT	56.2	55.6	47.1	74.1	85.7
MFGE8	SIM2(C-15)	59.4	72.2	47.1	75.9	71.4
MFGE8	MUC17	68.8	72.2	47.1	70.4	71.4
MFGE8	GAL3	59.4	61.1	47.1	74.1	85.7
MFGE8	ASPH(A-10)	62.5	66.7	41.2	72.2	71.4
MFGE8	CXCL12	56.2	55.6	52.9	74.1	71.4
Total number of Samples		**32**	**18**	**17**	**54**	**7**

[00914] The marker combinations were then assessed to identify those that best distinguish PCa from the 32 samples with BPH but without inflammatory conditions (see **Table 34).** Top performing detector-capture pairs are identified in **Table 43:**

PREOMICS EXHIBIT 1002
Page 1191 of 1861

Table 43: Discriminators of Cancer v Benign Prostate Disease

Detector	Capture	AUC
MFGE8	Integrin	0.730
MFGE8	NK-2R(C-21)	0.728
MFGE8	GAL3	0.728
MFGE8	Mammoglobin	0.721
MFGE8	MUC17	0.722
MFGE8	SIM2(C-15)	0.721
MFGE8	A33	0.715
MFGE8	EphA2	0.713
CD9, CD63, CD81	UNC93A	0.684
MFGE8	PBP	0.695
MFGE8	MUC1	0.690
MFGE8	CRP	0.674
CD9, CD63, CD81	S100-A4	0.689
CD81	CD81	0.688
MFGE8	HER3 (ErbB3)	0.667
CD9, CD63, CD81	ADAM10	0.686
MFGE8	Gro-alpha	0.684
MFGE8	CD9	0.684
MFGE8	NGAL	0.678
MFGE8	FRT	0.682

[00915] The marker combinations were then assessed to identify those that best distinguish PCa from inflammatory conditions (N=17). Top performing detector-capture pairs are identified in **Table 44:**

Table 44: Discriminators of Inflammatory Disease

Detector	Capture	AUC
Muc2	MISRII	0.559
CD81	Tsg101	0.676
Muc2	C-Bir	0.692
Muc2	MFG-E8	0.628
CD81	ALA	0.682
CD9, CD63, CD81	UNC93A	0.658
Muc2	TWEAK	0.628
CD81	PSMA Aptamer	0.634
Muc2	B7H3	0.574
Muc2	NGAL	0.664
CD81	GATA2	0.623

[00916] Other useful combinations included PCSA detector and EZH2 capture, and CD81 detector and PIMl capture.

[00917] Multiple panels of markers including those above can be combined to improve test performance. Using the data above, a multivariate model was used to examine the performance of panels using multiple capture agents. **FIG. 27** shows the results of two such panels using ROC curves. In **FIG.27A,** vesicles were captured using antibodies to mammaglobin, SIM2 and NK-2R and detected with PE-labeled anti MFG-E8 antibodies. The AUC was 0.90. In **FIG.27B,** vesicles were captured using antibodies to Integrin, NK-2R, and Gal3 and detected with PE-labeled anti MFG-E8 antibodies. **FIG. 27B** shows ROC curve generated by distinguishing 61 prostate cancer and 32 benign prostate samples. The AUC was 0.84.

PREOMICS EXHIBIT 1002
Page 1192 of 1861

Example 47: MicroRNA to Distinguish Cancer

[00918] The levels of various microRNAs were determined in prostate cancer and other samples as described in **Example 46.** MicroRNA levels were determined using Exiqon cards on concentrated vesicles from pooled patient plasma samples. Concentrated vesicles were isolated as described in **Example 20. Tables 45** and **46** show the top performing miRNA markers for distinguishing prostate cancer samples from non-prostate cancer samples (HGPIN, inflammatory and benign) using independent sets of pooled samples, ranked by AUC **(Table 45)** and p-value **(Table 46).** In **Table 46,** p-values were calculated using an unpaired t-test with Benjamini-Hochberg correction for multiple comparisons. **Table 47** shows the top performing miRNA markers for distinguishing prostate cancer samples from inflammatory disease samples.

Table 45: MicroRNA Discriminators of Prostate Cancer

miRNA Biomarker	AUC
miR-148a	0.678
miR-329	0.690
miR-9	0.678
miR-378*	0.648
miR-25	0.668
miR-614	0.643
miR-518c*	0.654
miR-378	0.651
miR-765	0.645
let-7f-2*	0.596
miR-574-3p	0.654
miR-497	0.650
miR-32	0.645
miR-379	0.611
miR-520g	0.612
miR-542-5p	0.647
miR-342-3p	0.647
miR-1206	0.564
miR-663	0.645
miR-222	0.641

Table 46: MicroRNA Discriminators of Prostate Cancer

miRNA Biomarker	P-value
hsa-miR-877*	0.033
hsa-miR-593	0.026
hsa-miR-595	0.021
hsa-miR-300	0.048
hsa-miR-324-5p	0.033
hsa-miR-548a-5p	0.021
hsa-miR-329	0.033
hsa-miR-550	0.003
hsa-miR-886-5p	0.033
hsa-miR-603	0.021
hsa-miR-490-3p	0.033
hsa-miR-938	0.033
hsa-miR-149	0.033
hsa-miR-150	0.033
hsa-miR-1296	0.005
hsa-miR-384	0.033
hsa-miR-487a	0.033
hsa-miRPlus-C1089	0.026

PREOMICS EXHIBIT 1002
Page 1193 of 1861

hsa-miR-485-3p	0.026
hsa-miR-525-5p	0.033

Table 47: MicroRNA Discriminators of Inflammatory Disease

miRNA Biomarker	AUC
miR-588	0.956
miR-1258	1.000
miR-16-2*	0.882
miR-938	0.905
miR-526b	0.746
miR-92b*	0.885
let-7d	0.740
miR-378*	0.854
miR-124	0.881
miR-376c	0.865
miR-26b	0.719
miR-1204	0.897
miR-574-3p	0.725
miR-195	1.000
miR-499-3p	0.751
miR-2110	0.760
miR-888	0.714

[00919] Performance of several individual microRNAs is shown in **FIG. 28. FIG. 28A** shows the levels of miR-614, which can distinguish prostate cancer from the other sample groups. miRs that distinguish inflammation from cancer are shown in **FIG. 28B** (miR-21 1) and **FIG. 28C** (miR-136). miRs that distinguish inflammation from cancer are shown in **FIG. 28D** (miR-149), **FIG. 28E** (miR-221 *), **FIG. 28F** (miR-329), and **FIG. 28G** (miR-26b).

Example 48: Circulating Microvesicles (cMVs) in Prostate Cancer Patient Samples

[00920] In this Example, cMVs are profiled in prostate cancer and related diseases. Methodology is similar to **Example 46** except that a different but overlapping panel of capture antibodies and a different but overlapping set of patient samples was used. Generally, capture agents (antibodies and/or aptamers) were tethered to fluorescently labeled microbeads and incubated with cMVs from patient plasma. The captured cMVs were detected with fluorescently labeled detector agents (antibodies and/or aptamers). Fluorescent signals are then used to compare levels of specific cMV populations in the patient samples. A total of 206 intended use samples are included in the study, including 92 cancers and 114 non-cancers. Patient characteristics are shown in **Table 48:**

Table 48: Patient Characteristics

Pathology Type	Number
Benign Prostate Disorder	54
Benign with Inflammation	31
High Grade Pin (HGPIN)	29
Cancer First Biopsy	79
Cancer Watchful Waiting	13

[00921] Capture and detector antibodies are shown in **Table 49:**

PREOMICS EXHIBIT 1002
Page 1194 of 1861

Table 49: Capture and Detector Antibodies

Antibody	Target Abbreviation	Clone	Vendor	Catalog Number
Anti filamin A alpha antibody	FLNA	4E10-1B2	Sigma-Aldrich	WH0002316M1
Anti human decorin antibody	DCRN	115402	R&D systems	MAB143
Anti Human Epidermal growth factor Receptor 3 antibody	HER 3 (ErbB3)	Polyclonal	US Biological	E3451-36A
Anti human Versican antibody	VCAN	255915	R&D systems	MAB3054
Anti-cluster of differentiation 9 antibody	CD9	209306	R&D Systems	MAB1880
Anti Galactose metabolism regulator 3 antibody	GAL3	B2C10	Santa Cruz	sc-32790
Anti cytidine and dCMP deaminase domain containing 1 antibody	CDADC1	1A2	Sigma-Aldrich	WH0081602M1
Anti-Human granulocyte macrophage colony stimulating factor antibody	GM-CSF	BVD2 23B6	Invitrogen	AHC2012
Anti epidermal growth factor antibody	EGFR	af231	BD biosciences	555996
Anti receptor activator of NFκB antibody	RANK	80704	R&D systems	MAB683
Anti-Chondroitin sulfate antibody	CSA	CS-56	abcam	ab11570
Anti Prostate specific membrane antibody	PSMA	LNI-17	Biolegend	342502
Anti chicken IgY antibody (NON-HPLC)	ChickenIgY	polyclonal	Abcam	ab50579
Anti Cluster of differentiation 276 antibody	B7H3	185504	R&D systems	MAB1027
Anti prostate cell surface antibody	PCSA	5 E 10	Inhouse	Inhouse
Anti cluster of differentiation 63 antibody	CD63	H5C6	BD pharmingen	556019
Anti CD3 antibody [OKT3]	CD3	Monoclonal	Abcam	ab86883
Anti Mucin 1, cell surface associated protein antibody	MUC1	Vu4H5	Santa Cruz	sc7313
Anti Transglutaminase-2 antibody	TGM2	2F4	Sigma Aldrich	WH0007052M10
Anti cluster of differentiation 81 antibody	CD81	JS-81	BD pharmingen	555675
Anti S100 calcium binding protein A4 antibody	S100-A4	1f12-1g7	Sigma aldrich	WH0006275M1
Anti Milk fat globule-EGF factor 8 protein antibody	MFG-E8	278918	R&D systems	MAB27671
Anti Integrin α5 (A-11) antibody	Integrin	A-11	Santacruz	sc-166665
Anti Neurokinin-A antibody	NK-2R(C-21)	Polyclonal	Santacruz	sc-14121
Anti Prostate specific antibody	PSA	BGN/PSA6	Novus Biologicals	NB100-66506
Anti Cluster of differntiation 24 antibody (Heat Stable antigen)	CD24	ml5	BD biosciences	bd 555426
Anti Tissue inhibitor of metallo proteinase-1 antibody	TIMP-1	4D12	Sigma-Aldrich	WH0007076M1
Anti human interleukin 6 unconjugated antibody	IL6 Unc	8H12	Invitrogen	AHC0762
Anti Prostatic binding protein antibody	PBP	2G2-1F1	Novus Biologicals	H00005037-M01
Anti proviral integration site antibody	PIM1	1C10	Novus Biologicals	H00005292-M08
Anti carbohydrate 19-9 antibody	CA-19-9	3H606	US Biological	C0075-13B
Anti TNF-related apoptosis-inducing ligand receptor 4 antibody	Trail-R4	104918	R&D systems	MAB633
Anti Matrixmetallo Proteinase 9 antibody	MMP9	SB15C	Novus biologicals	NBP1-28617
Anti prolactin Monoclonal antibody	PRL	6F11	Thermo Scientific Pierce	MA1-10597
Anti Ephrin-A receptor 2 antibody	EphA2	ka5h5	Santa Cruz	sc101377
Anti Tumor necrosis factor like weak inducer of	TWEAK	Poly	US biological	T9185-01

PREOMICS EXHIBIT 1002
Page 1195 of 1861

apoptosis				
Anti autoimmunogenic cancer/testis antigen	NY-ESO-1	6A 146	US biological	N8590-01
Anti mammaglobin A(C-16) antibody	Mammaglobin	polyclonal	Santa Cruz	sc-48328
Anti unc 93 homolog A antibody	UNC93A	113	Santa Cruz	scl35541
Anti glyco protein a33 antibody	A33	g20	Santa Cruz	sc33014
Anti Aurora Bkinase (serine/threonine -protein kinase 6) antibody	AURKB	6A6	Novus Biologicals	H00009212-M01A
Anti cluster of differentiation 41 antibody	CD41	PM6/248	Mybiosource	MBS2 10248
Anti X antigen family, member 1 antibody	XAGE-1	H-92	Santa cruz	sc-134820
Anti SAM pointed domain containing ets transcription factor antibody	SPDEF	4A5	Novus Biologicals	H00025803-M01
Anti Alpha-methylacyl-CoA racemase antibody	AMACR	1D8	Novus biologicals	H00023600-M02
Anti seprase antibody	seprase/FAP	427819	R&D	MAB3715
Anti Neutrophil gelatinase-associated lipocalin antibody	NGAL	hl30	Santa Cruz	sc50350
Anti Chemokine (C-X-C motif) ligand 12 antibody	CXCL12	7901 8	R&D systems	MAB350
Anti ferritin f31 antibody	FRT	F31	Santa Cruz	sc-5 1888
Anti carcino embryogenic antibody	CD66e CEA	Polyclonal	US Biological	C1300-08
Anti single minded protein 2 antibody	SIM2 (C-15)	Polyclonal	Santacruz	sc-8715
Anti Flagellin antibody	C-Bir	polyclonal	abeam	ab93713
Anti Six Transmembrane Epithelial Antigen of the Prostate 1 antibody	STEAP	polyclonal	Santacruz	sc-255 14
Anti Lens epithelium-derived growth factor	PSIP1/LEDGF	1C4	Novus biologicals	H0001 1168-M02
Anti Mucinl7, cell surface associated protein antibody	MUC17	cl9	Santa Cruz	sc32602
Anti Vascular Endothelial Growth Factor Receptor 2 antibody	hVEGFR2	89106	R&D systems	MAB3572
Anti Ets related gene antibody	ERG	Polyclonal	sigma aldrich	SAB2500363
Anti Mucin 2, cell surface associated protein antibody	MUC2	H-300	Santa Cruz	scl5334
Anti disintegrin and metalloproteinase domain 10 antibody	ADAM 10	163003	R&D systems	MAB1427
Anti Aspartyl/asparaginyl P-hydroxylase(AlO) antibody	ASPH (A- 10)	A-10	Santa Cruz	sc-271391
Anti carbohydrate antigen 125 antibody (MUC16)	CA125	8J453	US Biological	C0050-01D
Anti Human gro alpha antibody	Gro-alpha	Polyclonal	GeneTex	GTX10376
Anti tumor susceptibility gene 101 antibody	Tsg 101	Y16J	Santacruz	sc-101254
Anti synovial sarcoma X break point 2antibody	SSX2	1A4	Novus biologicals	H00006757-M01
Anti TNF-related apoptosis-inducing ligand receptor 4 antibody	Trail-R4			

[00922] Antibodies to five detector agents were used, comprising: 1) EpCam; 2) CD81 alone; 3) PCSA; 4) MUC2; and 5) MFG-E8. Combinations of detector agents along with microbe ad-tethered capture agents are shown in **Table 50.** In the table, the capture and/or detector agents comprised antibodies that recognize to the indicated targets unless noted as aptamers. The first row identifies the Detector agents. Beneath each detector is the list of capture agents used with the detector. Chicken IgY was run as a control.

Table 50: Capture and Detector Agent Combinations

EpCam	CD81	PCSA	MUC2	MFG-E8
FLNA	FLNA	FLNA	FLNA	FLNA
DCRN	DCRN	DCRN	DCRN	DCRN
HER 3 (ErbB3)	HER 3 (ErbB3)	HER 3 (ErbB3)	HER 3 (ErbB3)	HER 3 (ErbB3)
VCAN	VCAN	VCAN	VCAN	VCAN
CD9	CD9	CD9	CD9	CD9

PREOMICS EXHIBIT 1002
Page 1196 of 1861

GAL3	GAL3	GAL3	GAL3	GAL3
CDADC1	CDADC1	CDADC1	CDADC1	CDADC1
GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF
EGFR	EGFR	EGFR	EGFR	EGFR
RANK	RANK	RANK	RANK	RANK
CSA	CSA	CSA	CSA	CSA
PSMA	PSMA	PSMA	PSMA	PSMA
ChickenIgY	ChickenIgY	ChickenIgY	ChickenIgY	ChickenIgY
B7H3	B7H3	B7H3	B7H3	B7H3
PCSA	PCSA	PCSA	PCSA	PCSA
CD63	CD63	CD63	CD63	CD63
CD3	CD3	CD3	CD3	CD3
MUC1	MUC1	MUC1	MUC1	MUC1
TGM2	TGM2	TGM2	TGM2	TGM2
CD81	CD81	CD81	CD81	CD81
S100-A4	S100-A4	S100-A4	S100-A4	S100-A4
MFG-E8	MFG-E8	MFG-E8	MFG-E8	MFG-E8
Integrin	Integrin	Integrin	Integrin	Integrin
NK-2R(C-21)	NK-2R(C-21)	NK-2R(C-21)	NK-2R(C-21)	NK-2R(C-21)
PSA	PSA	PSA	PSA	PSA
CD24	CD24	CD24	CD24	CD24
TIMP-1	TIMP-1	TIMP-1	TIMP-1	TIMP-1
IL6 Unc	IL6 Unc	IL6 Unc	IL6 Unc	IL6 Unc
PBP	PBP	PBP	PBP	PBP
PIM1	PIM1	PIM1	PIM1	PIM1
CA-19-9	CA-19-9	CA-19-9	CA-19-9	CA-19-9
Trail-R4	Trail-R4	Trail-R4	Trail-R4	Trail-R4
MMP9	MMP9	MMP9	MMP9	MMP9
PRL	PRL	PRL	PRL	PRL
EphA2	EphA2	EphA2	EphA2	EphA2
TWEAK	TWEAK	TWEAK	TWEAK	TWEAK
NY-ESO-1	NY-ESO-1	NY-ESO-1	NY-ESO-1	NY-ESO-1
Mammaglobin	Mammaglobin	Mammaglobin	Mammaglobin	Mammaglobin
UNC93A	UNC93A	UNC93A	UNC93A	UNC93A
A33	A33	A33	A33	A33
AURKB	AURKB	AURKB	AURKB	AURKB
CD41	CD41	CD41	CD41	CD41
XAGE-1	XAGE-1	XAGE-1	XAGE-1	XAGE-1
SPDEF	SPDEF	SPDEF	SPDEF	SPDEF
AMACR	AMACR	AMACR	AMACR	AMACR
seprase/FAP	seprase/FAP	seprase/FAP	seprase/FAP	seprase/FAP
NGAL	NGAL	NGAL	NGAL	NGAL
CXCL12	CXCL12	CXCL12	CXCL12	CXCL12
FRT	FRT	FRT	FRT	FRT
CD66e CEA	CD66e CEA	CD66e CEA	CD66e CEA	CD66e CEA
SIM2 (C-15)	SIM2 (C-15)	SIM2 (C-15)	SIM2 (C-15)	SIM2 (C-15)
C-Bir	C-Bir	C-Bir	C-Bir	C-Bir
STEAP	STEAP	STEAP	STEAP	STEAP
PSIP1/LEDGF	PSIP1/LEDGF	PSIP1/LEDGF	PSIP1/LEDGF	PSIP1/LEDGF
MUC17	MUC17	MUC17	MUC17	MUC17
hVEGFR2	hVEGFR2	hVEGFR2	hVEGFR2	hVEGFR2
ERG	ERG	ERG	ERG	ERG
MUC2	MUC2	MUC2	MUC2	MUC2

PREOMICS EXHIBIT 1002
Page 1197 of 1861

ADAM10	ADAM10	ADAM10	ADAM10	ADAM10
ASPH (A-10)	ASPH (A-10)	ASPH (A-10)	ASPH (A-10)	ASPH (A-10)
CA125	CA125	CA125	CA125	CA125
Gro-alpha	Gro-alpha	Gro-alpha	Gro-alpha	Gro-alpha
Tsg 101	Tsg 101	Tsg 101	Tsg 101	Tsg 101
SSX2	SSX2	SSX2	SSX2	SSX2
Trail-R4	Trail-R4	Trail-R4	Trail-R4	Trail-R4

[00923] **Table 51** shows the top performing detector/capture combinations for distinguishing prostate cancer samples from all other samples. The levels of the detected vesicles were compared between these groups using a Wilcoxon test. P-values are shown in **Table 51:**

Table 51: MicroRNA Discriminators of Prostate Cancer

Detector	Capture	p-value
PCSA	A33	0.0002
Muc2	AURKB	0.0006
PCSA	Gro-alpha	0.0012
PCSA	TGM2	0.0030
PCSA	TWEAK	0.0054
PCSA	B7H3	0.0056
PCSA	FLNA	0.0082
PCSA	ADAM10	0.0104
EpCAM	Mammaglobin	0.0108
PCSA	NY-ESO-1	0.0114
PCSA	AMACR	0.0144
PCSA	S100-A4	0.0157
PCSA	Integrin	0.0180
PCSA	RANK	0.0188
PCSA	MMP9	0.0213
PCSA	EGFR	0.0225
PCSA	PSMA	0.0250
PCSA	hVEGFR2	0.0274
PCSA	GM-CSF	0.0291
EpCAM	PSIP1/LEDGF	0.0324
PCSA	CXCL12	0.0364
PCSA	CSA	0.0369
Muc2	PSIP1/LEDGF	0.0412
PCSA	CD41	0.0455
PCSA	SSX2	0.0513

[00924] Multi-biomarker panels were constructed from the capture/detector agents in **Table 50** on the plasma samples from patients in **Table 48.** Exemplary results are shown in **FIG. 29.** In **FIG. 29A,** the capture agents recognized AURKB, A33, CD63, Gro-alpha, and Integrin, and the detectors recognized MUC2, PCSA, and CD81 . The comparison was between the prostate cancer samples and all other samples. In this sample group, the AUC of the ROC curve was 0.8306, compared to only 0.59 for PSA. At the indicated point on the ROC curve, the sensitivity was 0.815 and the specificity was 0.737. By adjusting the classifier threshold used to distinguish cancer, the panel can be used to favor sensitivity or specificity as desired. In general, the more sensitive a test is for a disease, the higher its false-positive rate and the lower its specificity. A test with a higher specificity will usually sacrifice sensitivity by increasing its false-negative rate. This makes a highly sensitive test preferable for a screening examination, whereas a highly specific test may be preferred in a confirmatory role. In **FIG. 29A,** the threshold can be set such that the sensitivity is 95% and the specificity is 52%. This is the point on the curve

PREOMICS EXHIBIT 1002
Page 1198 of 1861

where sensitivity is 0.95 and 1 - specificity is 0.48. At this threshold, all four Gleason 8 and both Gleason 9 samples were classified correctly as prostate cancer, and 34 of 36 Gleason 7 were classified correctly and 44 of 48 Gleason 6 were classified correctly.

[00925] In **FIG. 29B,** the capture agents recognized AURKB, CD63, FLNA, A33, Gro-alpha, Integrin, CD24, SSX2, and SIM2, and the detectors recognized MUC2, PCSA, CD81, MFG-E8, and EpCam. The comparison was between the first biopsy prostate cancer samples (not the watchful waiting) and all other samples. In this sample group, the AUC of the ROC curve was 0.835, compared to only 0.60 for PSA. At the indicated point on the ROC curve, the sensitivity was 0.823 and the specificity was 0.737.

Example 49: MicroRNAs in Prostate Cancer Patient Samples

[00926] The levels of various microRNAs were determined in prostate cancer and other samples as described in **Example 48.** The samples comprised 2 μl aliquots of RNA extracted from concentrated vesicles from each of the samples listed in **Table 48.** Concentrated vesicles were isolated as described in **Example 20.** The samples were pooled by the groups listed in **Table 48.** MicroRNA levels were determined in triplicate Exiqon cards for each pool. Thirty-five microRNAs were identified that could distinguish between either prostate cancer and benign disease or prostate cancer and inflammatory samples, as shown in **Table 52.**

Table 52: MicroRNA Discriminators of Prostate Cancer

let-7d, miR-148a, miR-195, miR-25, miR-26b, miR-329, miR-376c, miR-574-3p, miR-888, miR-9, miR1204, miR-16-2*, miR-497, miR-588, miR-614, miR-765, miR92b*, miR-938, let-7f-2*, miR-300, miR-523, miR-525-5p, miR-1 182, miR-1244, miR-520d-3p, miR-379, let-7b, miR-125a-3p, miR-1296, miR-134, miR-149, miR-150, miR- 187, miR-32, miR-324-3p, miR-324-5p, miR-342-3p, miR-378, miR-378*, miR-384, miR-45 1, miR-455-3p, miR-485-3p, miR-487a, miR-490-3p, miR-502-5p, miR-548a-5p, miR-550, miR-562, miR-593, miR-593*, miR-595, miR-602, miR-603, miR-654-5p, miR-877*, miR-886-5p, miR-125a-5p, miR-140-3p, miR-192, miR- 196a, miR-21 10, miR-212, miR-222, miR-224*, miR-30b*, miR-499-3p, miR-505*

[00927] Various combinations of microRNAs in **Table 52** can be used to distinguish prostate cancer and benign diseases, including distinguishing prostate cancer from specific benign diseases such as inflammatory conditions.

Example 50: Combinations of Capture and Detection Agents for Prostate Cancer cMVs

[00928] In this Example, circulating microvesicles (cMVs) were profiled in plasma samples from prostate cancer and normal (non-prostate cancer) samples. Methodology was similar to **Examples 20-24.** cMVs from pools of PCa or normal samples were concentrated. Capture antibodies to the indicated vesicle antigens were tethered to fluorescently labeled microbeads and incubated with concentrated cMVs from patient plasma. The bead-bound and captured cMVs were labeled with PE labeled detector antibodies to the indicated vesicle antigens. Fluorescent signals were analyzed to determine levels of specific cMV populations in the patient samples.

[00929] **FIGs. 9-10** shows detection of cMVs captured with bead-tethered antibodies specific to general vesicle markers (CD9, CD63, CD81), prostate markers (PCSA, PSMA), and cancer markers (EpCam, B7H3). In those figures, the captured cMVs were simultaneously detected with labeled antibodies to the tetraspanins CD9, CD63, and CD81 . In this Example, vesicles were captured with bead-tethered antibodies specific to PCSA, PSMA, or B7H3. The captured cMVs were labeled with PE-labeled antibodies to PSMA, PCSA, B7H3, or the tetraspanins CD9, CD63, and CD81 . Results are shown in **FIG. 30A** for PCSA capture, **FIG. 30B** for PSMA capture, and **FIG. 30C** for B7H3 capture. In the figures, the Y-axis shows the average median fluorescence

PREOMICS EXHIBIT 1002
Page 1199 of 1861

intensity (MFI) of the detected antibodies. Samples as shown on the X-axis included PCa positive pools ("1 Pos Pool"), negative control pools from patients without PCa ("2 Neg Pool"), and a control blank ("Blank"). The detection agents as indicated on the X-axis include labeled antibodies to PSMA, PCSA or B7H3 individually, a cocktail of the antibodies to PSMA, PCSA, B7H3 ("cocktail"), or a cocktail of antibodies to the tetraspanins CD9, CD63, and CD81 ("VI - lets"). The results in **FIGs. 9, 10** and **30** demonstrate that the PCa vesicles can be distinguished from normal controls by capturing and/or detecting with various combinations of agents specific for general vesicle markers, prostate markers and cancer markers.

Example 51: Circulating Microvesicles (cMVs) in Prostate Cancer Patient Samples

[00930] In this Example, cMVs are profiled in prostate cancer and related diseases. Methodology is similar to **Examples 46** and **48** but a different but overlapping patient cohort was used. Generally, capture antibodies were tethered to fluorescently labeled microbeads and incubated with cMVs from patient plasma samples. The captured cMVs were detected with fluorescently labeled detector antibodies. Fluorescent signals are then used to compare levels of specific cMV populations in the patient samples. A total of 216 patient samples were included in the study, including 91 cancers and 125 non-cancers. All subjects had either a biopsy result of cancer and any subject with a negative result from a $\geq$ 10 core biopsy. Patient blood samples were clarified at 3000xg in a Labofuge centrifuge before cMVs were isolated from 1 mL of plasma by filtration (see **Example 20** for more details). Thirty samples that failed to pass quality measures were removed from further data analysis. Characteristics of 175 samples that passed quality controls are shown in **Table 53.** Eleven additional samples were collected from normals with no known prostate disorders but were not used in the comparisons in this Example.

Table 53: Patient Characteristics

Pathology Type	Number
Benign Prostate Disorder	48
Benign with Inflammation	27
High Grade Pin (HGPIN)	15
Prostatic atypia/Atypical small acinar proliferation (ASAP)	8
Cancer First Biopsy	71
Cancer Watchful Waiting	6

[00931] Capture and detector binding agents are shown in **Table 54:**

Table 54: Capture and Detector Antibodies

Binding Agent	Target	Vendor	Catalog#	Lot#
Anti filamin A alpha antibody	FLNA	Sigma-Aldrich	WH0002316M1	11165-51
Anti TNF-related apoptosis-inducing ligand receptor 4 antibody	Trail-R4	R&D systems	MAB633	DQQ0209121
Anti human Versican antibody	VCAN	R&D systems	MAB3054	UGW0209061
Anti-cluster of differentiation 9 antibody	CD9	R&D Systems	MAB1880	JOK0610081
Anti synovial sarcoma, X breakpoint 4 antibody	SSX4	Novus Biologicals	H00006759-MO2	11237-3E10
Anti CD3 antibody [OKT3]	CD3	Abcam	ab86883	GR52307-1
Anti carbohydrate 19-9 antibody	CA-19-9	US Biological	C0075-13B	L10122109
Anti membrane spanning 4A1 antibody	MS4A1	Sigma	WH0000931M1	091114-5C11
Anti carcino embryogenic antibody	CD66e CEA	US Biological	C1300-08	L11081075

PREOMICS EXHIBIT 1002
Page 1200 of 1861

Anti Mucin17, cell surface associated protein antibody	MUC17	Santa Cruz	sc32602	I0309
Anti epidermal growth factor antibody	EGFR	BD biosciences	555996	17563
Anti receptor activator of NFκB antibody	RANK	R&D systems	MAB683	EDV0209071
Anti-Chondroitin sulfate antibody	CSA	abcam	ab11570	GR18185-5
Anti Prostate specific membrane antibody	PSMA	Biolegend	342502	B132497
Anti human inactive complement component 3b antibody	iC3b	Thermo	MA1-82814	MG1439545
Anti chicken IgY antibody (NON-HPLC)	Antichicken IgY	Abcam	ab50579	GR41703-6
Anti Cluster of differentiation 276 antibody	B7H3	R&D systems	MAB1027	HPA0410081
Anti prostate cell surface antibody	PCSA	Inhouse	Inhouse	H10G006b
Anti cluster of differentiation 63 antibody	CD63	BD pharmingen	556019	82575
Anti Mucin 1, cell surface associated protein antibody	MUC1	Santa Cruz	sc7313	E2510
Anti Transglutaminase-2 antibody	TGM2	Sigma Aldrich	WH0007052M10	08309-2F4
Anti cluster of differentiation 81 antibody	CD81	BD pharmingen	555675	54545
Anti S100 calcium binding protein A4 antibody	S100-A4	Sigma aldrich	WH0006275M1	11222-S1/11210-S1
Anti Milk fat globule-EGF factor 8 protein antibody	MFG-E8	R&D systems	MAB27671	WQK0111031
Anti-Human granulocyte macrophage colony stimulating factor antibody	GM-CSF	Invitrogen	AHC2012	642599A
Anti Integrin α5 (A-11) antibody	Integrin	Santacruz	sc-166665	H0410
Anti Neurokinin-A antibody	NK-2R(C-21)	Santacruz	sc-14121	J0103
Anti Prostate specific antibody	PSA	Novus Biologicals	NB100-66506	300611
Anti Cluster of differntiation 24 antibody (Heat Stable antigen)	CD24	BD biosciences	bd 555426	5483
Anti Human Epidermal growth factor Receptor 3 antibody	HER3 (ErbB3)	US Biological	E3451-36A	L11092051
Anti Tissue inhibitor of metallo proteinase-1 antibody	TIMP-1	Sigma-Aldrich	WH0007076M1	11025-4D12
Anti human interleukin 6 unconjugated antibody	IL6 Unc	Invitrogen	AHC0762	706056A
Anti Prostatic binding protein antibody	PBP	Novus Biologicals	H00005037-M01	10264-S3/10236-2G2
Anti Apoptotic linked gene product 2 Interacting Protein X antibody	ALIX	Thermo scientific pierce	MA1-83977	MG1439546
Anti Matrixmetallo Proteinase 9 antibody	MMP9	Novus biologicals	NBP1-28617	K3205-V421
Anti prolactin Monoclonal antibody	PRL	Thermo Scientific Pierce	MA1-10597	MG1439591
Anti Ephrin-A receptor 2 antibody	EphA2	Santa Cruz	sc101377	K0409
Anti cytidine and dCMP deaminase domain containing 1 antibody	CDADC1	Sigma-Aldrich	WH0081602M1	11251-1A2
Anti Matrix metallo Proteinase 7 antibody	MMP7	Novus biologicals	NB300-1000	J10902
Anti c-reactive protein antibody	CRP	Abcam	ab13426	GR15824-6
Anti saccharomyces cerevisiae antibody	ASCA	abcam	ab19731	880975
Anti runt-related transcription factor 2 antibody	RUNX2	Sigma aldrich	WH0000860M1	10138-1D8
Anti Tumor necrosis factor like weak inducer of apoptosis	TWEAK	US biological	T9185-01	L11081013

PREOMICS EXHIBIT 1002
Page 1201 of 1861

Anti serpin peptidase inhibitor, clade B member 3 antibody	SERPINB3	Sigma aldrich	WH0006317M1	10155-2F5
Anti cytokeratin 19 fragment antibody	CYFRA21-1	MedixMab	102221	24594
Anti mammaglobin A(C-16) antibody	Mammaglobin	Santa Cruz	sc-48328	B2107
Anti Vascular endothelial growth factor A antibody	VEGF A	US Biological	V2110-05D	L10112413
Anti surfactant protein-C antibody	SPC	US Biological	U2575-03	L10100604
Anti Interleukin-1B antibody	IL-1B	Sigma Aldrich	WH0003553M1	10264-2A8
Anti tumor protein 53 antibody	p53	BioLegend	645802	B136322
Anti glyco protein a33 antibody	A33	Santa Cruz	sc33014	I0911
Anti Aurora Bkinase (serine/threonine-protein kinase 6) antibody	AURKB	Novus Biologicals	H00009212-M01A	11223-6A6
Anti cluster of differentiation 41 antibody	CD41	Mybiosource	MBS210248	n/a
Anti Chemokine (C-X-C motif) ligand 12 antibody	CXCL12	R&D systems	MAB350	COJ0510101
Anti X antigen family, member 1 antibody	XAGE	Santa cruz	sc-134820	B2210
Anti SAM pointed domain containing ets transcription factor antibody	SPDEF	Novus Biologicals	H00025803-M01	7285-4A5-00LcY6/11081-4A5
Anti Interleukin 8 antibody	IL8	Thermo scientific pierce	OMA1-03346	MG1439681
Anti B-cell novel protein1 antibody	BCNP	abcam	ab59781	GR49524-1
Anti Alpha-methylacyl-CoA racemase antibody	AMACR	Novus biological	H00023600-M02	11228-1D8
Anti human decorin antibody	DCRN	R&D systems	MAB143	EC10209061
Anti GATA binding protein 2 antibody	GATA2	Sigma-Aldrich	WH0002624M1	10271-2D11
Anti seprase antibody	seprase/FAP	R&D	MAB3715	CCHZ0109071
Anti Neutrophil gelatinase-associated lipocalin antibody	NGAL	Santa Cruz	sc50350	F0710
Anti Epithelial cellular adhesion molecule antibody	EpCAM	R&D systems	MAB 9601	UTT0911061
Anti Galactose metabolism regulator 3 antibody	GAL3	Santa Cruz	sc-32790	D0910
Anti proviral integration site antibody	PIM1	Novus Biologicals	H00005292-M08	11020-1C10
Anti tumor susceptibility gene 101 antibody	Tsg 101	Santacruz	sc-101254	I1310
Anti single minded protein 2 antibody	SIM2 (C-15)	Santacruz	sc-8715	G2810
Anti Flagellin antibody	C-Bir (Flagellin)	abcam	ab93713	GR35089-5
Anti Six Transmembrane Epithelial Antigen of the Prostate 1 antibody	STEAP	Santacruz	sc-25514	H2707/A0204
Anti heat shock protein antibody	HSP70	Biolegend	648002	B130984
Anti Vascular Endothelial Growth Factor Receptor 2 antibody	hVEGFR2	R&D systems	MAB3572	HHV0810011
Anti Ets related gene antibody	ERG	sigma aldrich	SAB2500363	7081P1
Anti autoimmunogenic cancer/testis antigen	NY-ESO-1	US biological	N8590-01	L11080550
Anti Mucin 2, cell surface associated protein antibody	MUC2	Santa Cruz	sc15334	B1811/G2111
Anti disintegrin and metalloproteinase domain 10 antibody	ADAM10	R&D systems	MAB1427	HZR0310021
Anti Aspartyl/asparaginyl β-hydroxylase(A10) antibody	ASPH (A-10)	Santa Cruz	sc-271391	B1411
Anti carbohydrate antigen 125 antibody (MUC16)	CA125	US Biological	C0050-01D	L11060368
Anti TNF-related apoptosis-inducing ligand receptor 2 antibody	TRAIL R2	Thermo scientific pierce	PA1-23497	MC1399147
Anti Human gro alpha antibody	Gro alpha	GeneTex	GTX10376	26629

PREOMICS EXHIBIT 1002
Page 1202 of 1861

Anti kallikrein-related peptidase 2 antibody	KLK2	Novus Biologicals	H00003817-M03	08130-3C5
Anti synovial sarcoma X break point 2 antibody	SSX2	Novus biologicals	H00006757-M01	11223-1A4

[00932] PE-labeled antibodies to five detector agents were used, comprising: 1) EpCam; 2) CD81 alone; 3) PCSA; 4) MUC2; and 5) MFG-E8. Combinations of detector agents along with microbead-tethered capture agents are shown in **Table 55.** In the table, the capture and/or detector agents comprised antibodies that recognize to the indicated targets unless noted as aptamers. The first row identifies the Detector agents. Beneath each detector is the list of capture agents used with the detector. Chicken IgY was run as a control.

Table 55: Capture and Detector Agent Combinations

EpCam	CD81	PCSA	MUC2	MFG-E8
FLNA	FLNA	FLNA	FLNA	FLNA
Trail-R4	Trail-R4	Trail-R4	Trail-R4	Trail-R4
VCAN	VCAN	VCAN	VCAN	VCAN
CD9	CD9	CD9	CD9	CD9
SSX4	SSX4	SSX4	SSX4	SSX4
CD3	CD3	CD3	CD3	CD3
CA-19-9	CA-19-9	CA-19-9	CA-19-9	CA-19-9
MS4A1	MS4A1	MS4A1	MS4A1	MS4A1
CD66e CEA	CD66e CEA	CD66e CEA	CD66e CEA	CD66e CEA
MUC17	MUC17	MUC17	MUC17	MUC17
EGFR	EGFR	EGFR	EGFR	EGFR
RANK	RANK	RANK	RANK	RANK
CSA	CSA	CSA	CSA	CSA
PSMA	PSMA	PSMA	PSMA	PSMA
iC3b	iC3b	iC3b	iC3b	iC3b
Chicken IgY	Chicken IgY	Chicken IgY	Chicken IgY	Chicken IgY
B7H3	B7H3	B7H3	B7H3	B7H3
PCSA	PCSA	PCSA	PCSA	PCSA
CD63	CD63	CD63	CD63	CD63
MUC1	MUC1	MUC1	MUC1	MUC1
TGM2	TGM2	TGM2	TGM2	TGM2
CD81	CD81	CD81	CD81	CD81
S100-A4	S100-A4	S100-A4	S100-A4	S100-A4
MFG-E8	MFG-E8	MFG-E8	MFG-E8	MFG-E8
GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF
Integrin	Integrin	Integrin	Integrin	Integrin
NK-2R(C-21)	NK-2R(C-21)	NK-2R(C-21)	NK-2R(C-21)	NK-2R(C-21)
PSA	PSA	PSA	PSA	PSA
CD24	CD24	CD24	CD24	CD24
HER3 (ErbB3)	HER3 (ErbB3)	HER3 (ErbB3)	HER3 (ErbB3)	HER3 (ErbB3)
TIMP-1	TIMP-1	TIMP-1	TIMP-1	TIMP-1
IL6 Unc	IL6 Unc	IL6 Unc	IL6 Unc	IL6 Unc
PBP	PBP	PBP	PBP	PBP
ALIX	ALIX	ALIX	ALIX	ALIX
MMP9	MMP9	MMP9	MMP9	MMP9
PRL	PRL	PRL	PRL	PRL
EphA2	EphA2	EphA2	EphA2	EphA2
CDADC1	CDADC1	CDADC1	CDADC1	CDADC1

PREOMICS EXHIBIT 1002
Page 1203 of 1861

MMP7	MMP7	MMP7	MMP7	MMP7
CRP	CRP	CRP	CRP	CRP
ASCA	ASCA	ASCA	ASCA	ASCA
RUNX2	RUNX2	RUNX2	RUNX2	RUNX2
TWEAK	TWEAK	TWEAK	TWEAK	TWEAK
SERPINB3	SERPINB3	SERPINB3	SERPINB3	SERPINB3
CYFRA21-1	CYFRA21-1	CYFRA21-1	CYFRA21-1	CYFRA21-1
Mammaglobin	Mammaglobin	Mammaglobin	Mammaglobin	Mammaglobin
VEGF A	VEGF A	VEGF A	VEGF A	VEGF A
SPC	SPC	SPC	SPC	SPC
IL-1B	IL-1B	IL-1B	IL-1B	IL-1B
p53	p53	p53	p53	p53
A33	A33	A33	A33	A33
AURKB	AURKB	AURKB	AURKB	AURKB
CD41	CD41	CD41	CD41	CD41
CXCL12	CXCL12	CXCL12	CXCL12	CXCL12
XAGE	XAGE	XAGE	XAGE	XAGE
SPDEF	SPDEF	SPDEF	SPDEF	SPDEF
IL8	IL8	IL8	IL8	IL8
BCNP	BCNP	BCNP	BCNP	BCNP
AMACR	AMACR	AMACR	AMACR	AMACR
DCRN	DCRN	DCRN	DCRN	DCRN
GATA2	GATA2	GATA2	GATA2	GATA2
seprase/FAP	seprase/FAP	seprase/FAP	seprase/FAP	seprase/FAP
NGAL	NGAL	NGAL	NGAL	NGAL
EpCAM	EpCAM	EpCAM	EpCAM	EpCAM
GAL3	GAL3	GAL3	GAL3	GAL3
PIM1	PIM1	PIM1	PIM1	PIM1
Tsg 101	Tsg 101	Tsg 101	Tsg 101	Tsg 101
SIM2 (C-15)	SIM2 (C-15)	SIM2 (C-15)	SIM2 (C-15)	SIM2 (C-15)
C-Bir (Flagellin)	C-Bir (Flagellin)	C-Bir (Flagellin)	C-Bir (Flagellin)	C-Bir (Flagellin)
STEAP	STEAP	STEAP	STEAP	STEAP
HSP70	HSP70	HSP70	HSP70	HSP70
hVEGFR2	hVEGFR2	hVEGFR2	hVEGFR2	hVEGFR2
ERG	ERG	ERG	ERG	ERG
NY-ESO-1	NY-ESO-1	NY-ESO-1	NY-ESO-1	NY-ESO-1
MUC2	MUC2	MUC2	MUC2	MUC2
ADAM10	ADAM10	ADAM10	ADAM10	ADAM10
ASPH (A-10)	ASPH (A-10)	ASPH (A-10)	ASPH (A-10)	ASPH (A-10)
CA125	CA125	CA125	CA125	CA125
TRAIL R2	TRAIL R2	TRAIL R2	TRAIL R2	TRAIL R2
Gro alpha	Gro alpha	Gro alpha	Gro alpha	Gro alpha
KLK2	KLK2	KLK2	KLK2	KLK2
SSX2	SSX2	SSX2	SSX2	SSX2

[00933] 25 μl of concentrated plasma was incubated with the antibody-conjugated microspheres for each detector/capture combination. In a parallel set of experiments, the anti-PCSA detector was also run with 3 μl of concentrated plasma was used for each capture. All samples were run in duplicate.

[00934] A number of different two-group comparisons were done to identify the capture/detector pair of markers (hereinafter "marker pairs") best able to discriminate the groups as outlined in the following tables. The

PREOMICS EXHIBIT 1002
Page 1204 of 1861

levels of the detected vesicles were compared between these groups using a non-parametric Kruskal-Wallace test corrected with Benjamini and Hochberg False Discovery Rate ("FDR") or Bonferroni's correction ("Bonf). Kruskal-Wallace is similar to analysis of variance with the data replaced by rank and is equivalent to the Mann-Whitney U test / Wilcoxon rank sum test when comparing two groups. Marker pairs with positive control data (PCa positive and negative pooled patient samples) that was indistinguishable from blank negative controls was excluded from further analysis. As another quality control measure, samples were excluded from analysis wherein the fluorescence values of vesicles captured using anti-CD9 antibody fall in the lower 5% of the data obtained using the CD81 detector. As the tetraspanins CD9 and CD81 are generally expressed on vesicles, this measure excludes sample with insufficient levels of vesicles. In the tables, detector "PCSA (25)" indicates samples where 25 µĩ of concentrated plasma was used with labeled anti-PCSA as a detector. Likewise, detector "PCSA (3)" indicates samples where 3 µĩ of concentrated plasma was used with labeled anti-PCSA as a detector.

[00935] **Table 56** shows the top performing detector/capture combinations for distinguishing prostate cancer (PCa+) samples from all other samples (PCA-). In this comparison, PCa+ is defined as any previous (i.e., watchful waiting) or current (i.e., first) positive biopsy and PCA- is defined as all other biopsy outcomes. Raw and corrected p-values are shown in **Table 56:**

Table 56: All Positive Biopsies v All Negative Biopsies

Detector	Capture	Effect size	Wilcoxon p-value	FDR	Bonf
Epcam	MMP7	0.8621	0.0000	0.0000	0.0000
PCSA (25)	MMP7	0.7953	0.0000	0.0000	0.0000
Epcam	BCNP	0.7840	0.0000	0.0000	0.0000
PCSA (25)	ADAM10	0.7589	0.0000	0.0000	0.0000
PCSA (25)	KLK2	0.7544	0.0000	0.0000	0.0000
PCSA (25)	SPDEF	0.7471	0.0000	0.0000	0.0000
PCSA (25)	IL-1B	0.7427	0.0000	0.0000	0.0000
PCSA (25)	EGFR	0.7361	0.0000	0.0000	0.0001
CD81	MMP7	0.7303	0.0000	0.0000	0.0002
PCSA (25)	CD9	0.7242	0.0000	0.0000	0.0003
PCSA (25)	EpCAM	0.7234	0.0000	0.0000	0.0004
PCSA (25)	PBP	0.7215	0.0000	0.0000	0.0004
PCSA (25)	P53	0.7199	0.0000	0.0000	0.0005
MFGE8	MMP7	0.7181	0.0000	0.0001	0.0013
PCSA (25)	SERPINB3	0.7091	0.0000	0.0001	0.0017
PCSA (25)	SSX4	0.6985	0.0000	0.0003	0.0052
PCSA (25)	SSX2	0.6967	0.0000	0.0003	0.0062
PCSA (25)	HER3 (ErbB3)	0.6967	0.0000	0.0003	0.0062
PCSA (25)	AURKB	0.6964	0.0000	0.0003	0.0064
PCSA (25)	BCNP	0.6934	0.0000	0.0004	0.0087
PCSA (25)	CD24	0.6920	0.0000	0.0005	0.0099
PCSA (25)	HSP70	0.6890	0.0000	0.0006	0.0133
PCSA (3)	BCNP	0.6888	0.0000	0.0006	0.0136
PCSA (25)	TGM2	0.6881	0.0000	0.0006	0.0146
PCSA (25)	CYFRA21-1	0.6862	0.0000	0.0007	0.0176

[00936] In **Table 57,** a subset of PCa+ and PCa- samples was compared. The samples met the following criteria: 1) Positive biopsy or negative biopsy with $\geq$ 10 cores; 2) 40 $\leq$ age $\leq$ 75; 3) 0 $\leq$ serum PSA (ng/ml) $\leq$ 10; and 4) no previous biopsies (either positive or negative). The sample cohort meeting this criteria is referred to as the "restricted sample set."

PREOMICS EXHIBIT 1002
Page 1205 of 1861

Table 57: Restricted Positive Biopsies v Negative Biopsies

Detector	Capture	Effect size	Wilcoxon p-value	FDR	Bonf
Epcam	MMP7	0.8947	0.0000	0.0000	0.0000
Epcam	BCNP	0.8310	0.0000	0.0000	0.0000
PCSA (25)	MMP7	0.8125	0.0000	0.0000	0.0000
PCSA (25)	ADAM10	0.7647	0.0000	0.0000	0.0002
CD81	MMP7	0.7568	0.0000	0.0001	0.0004
PCSA (25)	SPDEF	0.7510	0.0000	0.0001	0.0007
PCSA (25)	IL-1B	0.7505	0.0000	0.0001	0.0007
PCSA (25)	EGFR	0.7384	0.0000	0.0003	0.0022
PCSA (25)	KLK2	0.7358	0.0000	0.0003	0.0027
PCSA (25)	p53	0.7244	0.0000	0.0007	0.0074
PCSA (25)	EpCAM	0.7236	0.0000	0.0007	0.0080
PCSA (25)	CD9	0.7227	0.0000	0.0007	0.0087
PCSA (3)	BCNP	0.7209	0.0000	0.0007	0.0101
MFGE8	MMP7	0.7286	0.0000	0.0007	0.0104
PCSA (25)	AURKB	0.7174	0.0000	0.0009	0.0136
PCSA (25)	BCNP	0.7110	0.0001	0.0014	0.0230
PCSA (25)	PBP	0.7070	0.0001	0.0019	0.0319
PCSA (25)	CSA	0.7024	0.0001	0.0025	0.0459
CD81	BCNP	0.7007	0.0001	0.0028	0.0527
Muc2	PRL	0.6986	0.0001	0.0030	0.0617
PCSA (25)	SERPINB3	0.6984	0.0001	0.0030	0.0629
PCSA (25)	ASCA	0.6979	0.0001	0.0030	0.0654
Muc2	TIMP-1	0.6950	0.0002	0.0036	0.0818
PCSA (25)	SSX2	0.6926	0.0002	0.0041	0.0981
PCSA (25)	CA-19-9	0.6913	0.0002	0.0043	0.1079

[00937] In **Table 58,** a second subset of PCa+ and PCa- samples was compared. The samples met the following criteria: 1) Positive biopsy or negative biopsy with $\geq$ 10 cores; 2) 40 $\leq$ age $\leq$ 75; 3) 0 $\leq$ serum PSA (ng/ml) $\leq$ 10; and 4) no previous positive biopsies (but may have had previous negative biopsy). Note the criteria 4) differs from the cohort directly above.

Table 58: Restricted Positive Biopsies v Negative Biopsies

Detector	Capture	Effect size	Wilcoxon p-value	FDR	Bonf
Epcam	MMP7	0.8975	0.0000	0.0000	0.0000
Epcam	BCNP	0.8278	0.0000	0.0000	0.0000
PCSA (25)	MMP7	0.8252	0.0000	0.0000	0.0000
PCSA (25)	ADAM10	0.7772	0.0000	0.0000	0.0000
PCSA (25)	SPDEF	0.7656	0.0000	0.0000	0.0001
PCSA (25)	IL-1B	0.7614	0.0000	0.0000	0.0001
CD81	MMP7	0.7568	0.0000	0.0000	0.0002
PCSA (25)	EGFR	0.7538	0.0000	0.0000	0.0002
PCSA (25)	KLK2	0.7514	0.0000	0.0000	0.0003
PCSA (25)	EpCAM	0.7403	0.0000	0.0001	0.0008
PCSA (25)	p53	0.7398	0.0000	0.0001	0.0009
PCSA (25)	CD9	0.7373	0.0000	0.0001	0.0011
PCSA (3)	BCNP	0.7319	0.0000	0.0001	0.0018
MFGE8	MMP7	0.7385	0.0000	0.0002	0.0022
PCSA (25)	BCNP	0.7290	0.0000	0.0002	0.0024
PCSA (25)	AURKB	0.7279	0.0000	0.0002	0.0027
PCSA (25)	PBP	0.7255	0.0000	0.0002	0.0033
PCSA (25)	ASCA	0.7168	0.0000	0.0004	0.0074
Muc2	PRL	0.7161	0.0000	0.0004	0.0079

PREOMICS EXHIBIT 1002
Page 1206 of 1861

PCSA (25)	CSA	0.7154	0.0000	0.0004	0.0084
PCSA (25)	SERPINB3	0.7141	0.0000	0.0004	0.0094
PCSA (25)	SSX2	0.7124	0.0000	0.0005	0.01 10
PCSA (25)	CYFRA21-1	0.7102	0.0000	0.0006	0.0133
PCSA (25)	HER3 (ErbB3)	0.7093	0.0000	0.0006	0.0143
PCSA (25)	CA-19-9	0.7073	0.0000	0.0007	0.0170

[00938] **Table 59** shows the results when comparing newly identified PCa+ versus all PCA- samples. This comparison excludes the watchful waiting samples.

Table 59: Newly Identified Positive Biopsies v Negative Biopsies

Detector	Capture	Effect size	Wilcoxon p-value	FDR	Bonf
Epcam	MMP7	0.8767	0.0000	0.0000	0.0000
PCSA (25)	MMP7	0.8108	0.0000	0.0000	0.0000
Epcam	BCNP	0.8018	0.0000	0.0000	0.0000
PCSA (25)	ADAM10	0.7764	0.0000	0.0000	0.0000
PCSA (25)	KLK2	0.7672	0.0000	0.0000	0.0000
PCSA (25)	SPDEF	0.7644	0.0000	0.0000	0.0000
PCSA (25)	IL-1B	0.7576	0.0000	0.0000	0.0000
PCSA (25)	EGFR	0.7525	0.0000	0.0000	0.0000
PCSA (25)	CD9	0.7410	0.0000	0.0000	0.0001
PCSA (25)	EpCAM	0.7367	0.0000	0.0000	0.0001
PCSA (25)	p53	0.7366	0.0000	0.0000	0.0001
PCSA (25)	PBP	0.7360	0.0000	0.0000	0.0002
CD81	MMP7	0.7350	0.0000	0.0000	0.0002
PCSA (25)	SERPINB3	0.7208	0.0000	0.0001	0.0008
MFGE8	MMP7	0.7231	0.0000	0.0001	0.0013
PCSA (25)	SSX2	0.7151	0.0000	0.0001	0.0015
PCSA (25)	HER3 (ErbB3)	0.7139	0.0000	0.0001	0.0017
PCSA (25)	SSX4	0.7098	0.0000	0.0001	0.0026
PCSA (25)	AURKB	0.7091	0.0000	0.0001	0.0028
PCSA (25)	BCNP	0.7071	0.0000	0.0002	0.0034
PCSA (25)	TGM2	0.7052	0.0000	0.0002	0.0041
PCSA (25)	CD24	0.7049	0.0000	0.0002	0.0043
PCSA (3)	BCNP	0.7028	0.0000	0.0002	0.0052
PCSA (25)	HSP70	0.7027	0.0000	0.0002	0.0053
PCSA (25) 43	MMP9	0.7022	0.0000	0.0002	0.0056

[00939] The analysis for the results in **Table 60** was high-risk of PCa vs. low-risk of PCa samples. High risk is defined as postive cancer biopsy as well as HGPEST and ATYPIA/ASAP. Low risk samples are the remainder.

Table 60: High-risk of PCa vs. Low-risk of PCa

Detector	Capture	Effect size	Wilcoxon p-value	FDR	Bonf
Epcam	MMP7	0.8269	0.0000	0.0000	0.0000
Epcam	BCNP	0.7399	0.0000	0.0000	0.0000
PCSA (25)	MMP7	0.7284	0.0000	0.0001	0.0002
PCSA (25)	KLK2	0.7222	0.0000	0.0001	0.0004
PCSA (25)	SPDEF	0.7025	0.0000	0.0006	0.0031
PCSA (25)	ADAM10	0.6988	0.0000	0.0007	0.0046
CD81	MMP7	0.6982	0.0000	0.0007	0.0049
PCSA (25)	SSX2	0.6929	0.0000	0.0010	0.0083
PCSA (25)	PBP	0.6925	0.0000	0.0010	0.0087
PCSA (25)	EpCAM	0.6914	0.0000	0.0010	0.0096
PCSA (25)	p53	0.6857	0.0000	0.0015	0.0169

PREOMICS EXHIBIT 1002
Page 1207 of 1861

Muc2	MMP7	0.6847	0.0000	0.0015	0.0186
Muc2	PRL	0.6845	0.0000	0.0015	0.0190
PCSA (25)	CD24	0.6828	0.0001	0.0016	0.0223
PCSA (25)	MMP9	0.6818	0.0001	0.0016	0.0245
PCSA (25)	EGFR	0.6781	0.0001	0.0022	0.0344
PCSA (25)	IL-1B	0.6767	0.0001	0.0023	0.0394
PCSA (25)	CD9	0.6735	0.0001	0.0029	0.0527
PCSA (25)	SSX4	0.6720	0.0001	0.0030	0.0600
MFGE8	TIMP-1	0.6759	0.0001	0.0030	0.0604
PCSA (25)	HER3 (ErbB3)	0.6713	0.0001	0.0031	0.0641
MFGE8	MMP7	0.6740	0.0002	0.0032	0.0714
PCSA (25)	HSP70	0.6613	0.0004	0.0067	0.1534
PCSA (25)	CYFRA21-1	0.6600	0.0004	0.0070	0.1713
MFGE8	BCNP	0.6633	0.0004	0.0070	0.1761

[00940] The analysis for the results in **Table 61** consisted of all PCA+ samples compared to inflammation positive samples. All other outcomes were excluded.

Table 61: Prostate Cancer v Prostate Inflammatory Conditions

Detector	Capture	Effect size	Wilcoxon p-value	FDR	Bonf
EpCam	MMP7	0.8196	0.0000	0.0007	0.0007
EpCam	BCNP	0.7914	0.0000	0.0026	0.0052
PCSA (25)	IL-1B	0.7579	0.0001	0.0130	0.0470
PCSA (25)	ADAM10	0.7562	0.0001	0.0130	0.0520
PCSA (25)	KLK2	0.7319	0.0005	0.0386	0.2177
PCSA (25)	EGFR	0.7308	0.0005	0.0386	0.2313
PCSA (25)	SPDEF	0.7256	0.0007	0.0439	0.3076
PCSA (25)	CD9	0.7232	0.0008	0.0439	0.3513
MFGE8	TIMP-1	0.7168	0.0013	0.0619	0.5574
PCSA (25)	p53	0.7048	0.0021	0.0921	0.9213
PCSA (25)	MMP7	0.7021	0.0024	0.0959	1.0000
PCSA (25)	PBP	0.6966	0.0032	0.1148	1.0000

[00941] The analysis for the results in **Table 62** consisted of all PCA+ samples compared to "benign" prostate conditions, where "benign" is defined as a negative biopsy without inflammatory condition.

Table 62: Prostate Cancer v Non-inflammatory Benign Prostate Conditions

Detector	Capture	Effect size	Wilcoxon p-value	FDR	Bonf
Epcam	MMP7	0.9161	0.0000	0.0000	0.0000
PCSA (25)	MMP7	0.8465	0.0000	0.0000	0.0000
Epcam	BCNP	0.7964	0.0000	0.0000	0.0000
PCSA (25)	KLK2	0.7864	0.0000	0.0000	0.0001
CD81	MMP7	0.7714	0.0000	0.0000	0.0002
PCSA (25)	SPDEF	0.7679	0.0000	0.0001	0.0003
PCSA (25)	EpCAM	0.7648	0.0000	0.0001	0.0004
PCSA (25)	SSX2	0.7544	0.0000	0.0001	0.0012
PCSA (25)	ADAM10	0.7541	0.0000	0.0001	0.0012
MFGE8	MMP7	0.7605	0.0000	0.0001	0.0013
PCSA (25)	PBP	0.7474	0.0000	0.0002	0.0022
PCSA (25)	SSX4	0.7441	0.0000	0.0002	0.0029
Muc2	MMP7	0.7430	0.0000	0.0002	0.0032
PCSA (25)	p53	0.7391	0.0000	0.0003	0.0045
PCSA (25)	EGFR	0.7344	0.0000	0.0004	0.0067
PCSA (25)	CD24	0.7344	0.0000	0.0004	0.0067

PREOMICS EXHIBIT 1002
Page 1208 of 1861

PCSA (25)	MMP9	0.7324	0.0000	0.0005	0.0079
PCSA (25)	SERPINB3	0.7295	0.0000	0.0005	0.0101
PCSA (25)	HSP70	0.7295	0.0000	0.0005	0.0101
PCSA (25)	CD3	0.7256	0.0000	0.0007	0.0137
PCSA (25)	IL-1B	0.7245	0.0000	0.0007	0.015 1
PCSA (25)	CD9	0.7221	0.0000	0.0008	0.01 82
PCSA (25)	HER3 (ErbB3)	0.7198	0.0001	0.0010	0.0220
PCSA (25)	TIMP	0.7171	0.0001	0.001 1	0.0270
PCSA (25)	CYFRA21-1	0.7163	0.0001	0.0012	0.0289

[00942] **Table 63** shows the results of comparing all PCA+ samples with all high-grade prostatic intraepithelial neoplasia (HGPEM) samples.

Table 63: Prostate Cancer v HGPIN

Detector	Capture	Effect size	Wilcoxon p-value	FDR	Bonf
Epcam	MMP7	0.7945	0.0000	0.0074	0.0143
PCSA (25)	MMP7	0.7939	0.0000	0.0074	0.0148
PCSA (25)	ADAM10	0.7727	0.0001	0.0174	0.0523
PCSA (25)	IL-1B	0.7644	0.0002	0.0210	0.0840
Epcam	BCNP	0.7484	0.0005	0.0329	0.2009
PCSA (25)	EGFR	0.7458	0.0005	0.0329	0.2300
PCSA (3)	BCNP	0.7458	0.0005	0.0329	0.2300
PCSA (25)	CD9	0.7298	0.0012	0.0651	0.5206
PCSA (25)	SPDEF	0.7273	0.0014	0.0656	0.5906
Epcam	TRAIL R2	0.7209	0.0018	0.0732	0.8055
PCSA (25)	AURKB	0.7209	0.0018	0.0732	0.8055
PCSA (25)	SERPINB3	0.7164	0.0023	0.0802	0.9963
Epcam	NGAL	0.7154	0.0024	0.0802	1.0000
PCSA (25)	seprase/FAP	0.7113	0.0029	0.0837	1.0000
PCSA (25)	KLK2	0.7113	0.0029	0.0837	1.0000
PCSA (25)	ERG	0.7100	0.0031	0.0837	1.0000
PCSA (25)	TRAIL R2	0.7087	0.0033	0.0837	1.0000
PCSA (25)	STEAP	0.7068	0.0036	0.0862	1.0000
PCSA (25)	EpCAM	0.6997	0.0049	0.0983	1.0000
CD81	MMP7	0.6991	0.0050	0.0983	1.0000
MFGE8	MMP7	0.7042	0.0051	0.0983	1.0000
PCSA (25)	TGM2	0.6978	0.0053	0.0983	1.0000
PCSA (25)	CRP	0.6972	0.0054	0.0983	1.0000
PCSA (25)	CD81	0.6959	0.0057	0.0983	1.0000
PCSA (25)	p53	0.6959	0.0057	0.0983	1.0000

[00943] The results in **Table 64** were obtained by comparing bins of total Gleason score for subjects with cancer biopsy. Samples were grouped by low Gleason (<5), intermediate Gleason **(6-9)** and high Gleason (>10). P-values were not corrected due to small sample sizes.

Table 64: Gleason Score Comparison

Detector	Capture	Effect size	KW p-value
CD81	CD41	8.1729	0.0043
CD81	VCAN	7.3313	0.0068
CD81	MUC1	7.1483	0.0075
CD81	Integrin	7.0934	0.0077
Epcam	EpCAM	6.8867	0.0087
CD81	Gro alpha	6.8058	0.0091
CD81	PIM1	6.4976	0.0108

PREOMICS EXHIBIT 1002
Page 1209 of 1861

CD81	GM-CSF	6.4846	0.0109
CD81	TRAIL R2	6.3732	0.01 16
CD81	RUNX2	5.9838	0.0144
CD81	EpCAM	5.8523	0.0156
CD81	PSMA	5.8309	0.0157
CD81	TWEAK	5.715 1	0.0168
CD81	EphA2	5.6480	0.0175
CD81	CD24	5.5969	0.0180
CD81	S100-A4	5.5323	0.01 87
CD81	SPC	5.4956	0.0191
Epcam	EphA2	5.4743	0.0193
CD81	AURKB	5.4580	0.0195
CD81	IL-1B	5.4358	0.0197
CD81	ERG	5.3871	0.0203
CD81	EGFR	5.2719	0.0217
CD81	ADAM 10	5.2376	0.0221

[00944] In **Table 65,** results were obtained by comparing groups of samples in the following categories: 1) benign; 2) inflammation; 3) ATYPIA/ASAP/HGPIN; 4) PCA+, total Gleason score = 6-9.

Table 65: Clinical Category Comparison

Detector	Capture	Effect size	KW p-value	FDR	Bonf
Epcam	MMP7	52.6024	0.0000	0.0000	0.0000
PCSA (25)	MMP7	34.1291	0.0000	0.0000	0.0000
Epcam	BCNP	31.4758	0.0000	0.0000	0.0000
CD81	MMP7	26.3295	0.0000	0.0000	0.0001
PCSA (25)	ADAM10	25.7130	0.0000	0.0000	0.0002
PCSA (25)	EpCAM	25.2041	0.0000	0.0000	0.0002
PCSA (25)	SPDEF	25.1335	0.0000	0.0000	0.0002
PCSA (25)	IL-1B	23.5724	0.0000	0.0001	0.0005
PCSA (25)	PBP	22.2470	0.0000	0.0001	0.0010
PCSA (25)	EGFR	21.0674	0.0000	0.0002	0.0019
PCSA (25)	SSX4	20.1831	0.0000	0.0003	0.0031
PCSA (25)	SSX2	19.4117	0.0000	0.0004	0.0046
PCSA (25)	p53	19.0491	0.0000	0.0004	0.0056
PCSA (25)	KLK2	18.8417	0.0000	0.0004	0.0062
PCSA (25)	MMP9	18.3870	0.0000	0.0005	0.0079
PCSA (25)	CD9	18.1835	0.0000	0.0005	0.0088
PCSA (25)	SERPINB3	17.5771	0.0000	0.0007	0.0121
PCSA (25)	HSP70	17.2052	0.0000	0.0008	0.0147
Epcam	p53	16.1627	0.0001	0.0013	0.0254
PCSA (25)	CSA	15.8084	0.0001	0.0015	0.0306
PCSA (25)	HER3 (ErbB3)	15.5570	0.0001	0.0016	0.0350
Epcam	EpCAM	15.5062	0.0001	0.0016	0.0359
MFGE8 47	MMP7	15.4614	0.0001	0.0016	0.0368
PCSA (25) 34	CD24	15.1291	0.0001	0.0018	0.0439
PCSA (25) 53	CYFRA21-1	14.9992	0.0001	0.0019	0.0470

[00945] In **Table 66,** results are shown for analysis of PCa+ subjects with total Gleason score $\geq$ 7 compared to PCa+ subjects with Gleason score of 6 and PCa- subjects.

Table 66: High Gleason v Others

Detector	Capture	Effect size	Wilcoxon p-value	FDR	Bonf
Epcam	EpCAM	0.7697	0.0000	0.0004	0.0010

PREOMICS EXHIBIT 1002
Page 1210 of 1861