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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/381,888	07/21/2021	Douglas A. Amorese	NUGN-001/04US 34079/550	6188
21710	7590	08/15/2023	EXAMINER	
BROWN RUDNICK LLP ONE FINANCIAL CENTER BOSTON, MA 02111			STRZELECKA, TERESA E	
			ART UNIT	PAPER NUMBER
			1637	
			NOTIFICATION DATE	DELIVERY MODE
			08/15/2023	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):

ip@brownrudnick.com
usactions@brownrudnick.com



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APPLICATION NO.	ISSUE DATE	PATENT NO.
17/381,888	15-Aug-2023	11725241

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EGRANT NOTIFICATION

Your electronic patent grant (eGrant) is now available, which can be accessed via Patent Center at <https://patentcenter.uspto.gov>

The electronic patent grant is the official patent grant under 35 U.S.C. 153. For more information, please visit <https://www.uspto.gov/electronicgrants>



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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/381,888	08/15/2023	11725241	NUGN-001/04US 34079/550	6188

21710 7590 07/26/2023

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at <https://patentcenter.uspto.gov/>. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

The USPTO is implementing electronic patent issuance with a transition period, during which period the USPTO will mail a ceremonial paper copy of the electronic patent grant to the correspondence address of record. Additional copies of the patent (i.e., certified and presentation copies) may be ordered for a fee from the USPTO's Certified Copy Center at <https://certifiedcopycenter.uspto.gov/index.html>. The Certified Copy Center may be reached at (800)972-6382.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 187 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

INVENTOR(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional inventors):

Douglas A. Amorese, Los Altos, CA;
Jonathan Scolnick, San Francisco, CA;
Ben Schroeder, San Mateo, CA;

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Tecan Genomics, Inc., Redwood City, CA;

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 USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

PART B-FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

By fax, send to: (571) 273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Brown Rudnick LLP
One Financial Center
Boston, Massachusetts 02111

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

Michelle Aiello	(Typed or printed name)
/Michelle Aiello/	(Signature)
June 26, 2023	(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
17/381,888	07/21/2021	Douglas A. Amorese	NUGN-001/04US 34079/550	6188

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ

APPLN: TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1,200.00	\$0.00	\$0.00	\$1,200.00	06/27/2023

EXAMINER	ART UNIT	CLASS-SUBCLASS
Strzelecka, Teresa E.	1637	506-004000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
- (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Brown Rudnick LLP
2. Adam M. Schoen
- 3.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE or COUNTRY)

Tecan Genomics, Inc.

Redwood City, California

Please check the appropriate assignee category or categories (will not be printed on the patent):

☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees Submitted: ☒ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment (Please first reapply any previously paid fee shown above):

- ☒ Electronic Payment via Patent Center or EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)
- ☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 50-0369

5. Change of Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29.
- ☐ Applicant asserting small entity status. See 37 CFR 1.27.
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid Certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken as a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature

/Adam M. Schoen/

Date June 26, 2023

Typed or printed name

Adam M. Schoen

Registration No.

58,576

Electronic Patent Application Fee Transmittal

Application Number:	17381888			
Filing Date:	21-Jul-2021			
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ			
First Named Inventor/Applicant Name:	Douglas A. Amorese			
Filer:	Adam M Schoen/Michelle Aiello			
Attorney Docket Number:	NUGN-001/04US 34079/550			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	1501	1	1200	1200

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				1200

Electronic Acknowledgement Receipt

EFS ID:	48205348
Application Number:	17381888
International Application Number:	
Confirmation Number:	6188
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ
First Named Inventor/Applicant Name:	Douglas A. Amorese
Customer Number:	21710
Filer:	Adam M Schoen/Michelle Aiello
Filer Authorized By:	Adam M Schoen
Attorney Docket Number:	NUGN-001/04US 34079/550
Receipt Date:	26-JUN-2023
Filing Date:	21-JUL-2021
Time Stamp:	16:55:27
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$ 1200
RAM confirmation Number	E20236PG55405110
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

QIAGEN Exhibit 1012.0008

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	NUGN-001-04US-Issue-Fee-Transmittal.pdf	37011	no	1
			6d4dbfdea9637f06397af172d2012606de961617		
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	37953	no	2
			bf4f5736a7a62042cd7eb7f4213a0f023ed62188		
Warnings:					
Information:					
Total Files Size (in bytes):			74964		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					



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NOTICE OF ALLOWANCE AND FEE(S) DUE

21710 7590 03/27/2023
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER	
STRZELECKA, TERESA E	
ART UNIT	PAPER NUMBER

1637

DATE MAILED: 03/27/2023

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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17/381,888

07/21/2021

Douglas A. Amorese

NUGN-001/04US
34079/550

6188

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1200	\$0.00	\$0.00	\$1200	06/27/2023

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

21710 7590 03/27/2023
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/381,888	07/21/2021	Douglas A. Amorese	NUGN-001/04US 34079/550	6188

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1200	\$0.00	\$0.00	\$1200	06/27/2023

EXAMINER	ART UNIT	CLASS-SUBCLASS
STRZELECKA, TERESA E	1637	506-004000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

1 _____

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☐ Electronic Payment via Patent Center or EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

☐ Applicant asserting small entity status. See 37 CFR 1.27

☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____



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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/381,888	07/21/2021	Douglas A. Amorese	NUGN-001/04US	6188
21710	7590	03/27/2023	34079/550	
BROWN RUDNICK LLP ONE FINANCIAL CENTER BOSTON, MA 02111			EXAMINER STRZELECKA, TERESA E	
			ART UNIT	PAPER NUMBER
			1637	
DATE MAILED: 03/27/2023				

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. 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 30 minutes 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, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

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) 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 disclosure of these records is required by the Freedom of Information Act.
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 inspection 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.

Notice of Allowability	Application No. 17/381,888	Applicant(s) Amorese et al.	
	Examiner TERESA E STRZELECKA	Art Unit 1637	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☐ This communication is responsive to _____.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 22-37. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☐ All b) ☐ Some* c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. _____.

3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☒ Notice of References Cited (PTO-892) 2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date <u>8/4/22</u> . 3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____. 4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.	5. ☐ Examiner's Amendment/Comment 6. ☒ Examiner's Statement of Reasons for Allowance 7. ☐ Other _____.
---	--

/TERESA E STRZELECKA/ Primary Examiner, Art Unit 1637	
--	--

DETAILED ACTION

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.
2. Claims 22-37 are pending and under consideration.

Priority

3. The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of 35 U.S.C. 112(a) or the first paragraph of pre-AIA 35 U.S.C. 112, except for the best mode requirement. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994)

The disclosure of the prior-filed applications, Application No. 16/743,604 (filed January 15, 2020); Application No. 15/406,002 (filed January 13, 2017); Application No. 14/540,917 (filed November 13, 2014) and Application No. 61/903,826 (filed November 13, 2013), fail to provide adequate support or enablement in the manner provided by 35 U.S.C. 112(a) or pre-AIA 35 U.S.C. 112, first paragraph for one or more claims of this application.

Specifically, claim 22 of the instant application is drawn to a method for detecting duplicate sequencing reads, the method comprising:

obtaining amplicons each comprising an amplified fragment of a nucleic acid with an appended adaptor, wherein each adaptor comprises an identifier site comprising a plurality of nucleotides unique to the amplified fragment;

sequencing the amplicons to generate sequence reads that include identifier and target sequences; and

identifying sequence reads with identical identifier and target sequences as duplicates.

However, there is no support in the specification of the instant application and disclosures in the parent cases for the limitation of an adapter comprising an identifier site comprising a plurality of nucleotides unique to the amplified fragment. The claim requires that each fragment of the nucleic acid has a unique identifier specific to the fragment. Let us consider Example 1, in which 500 ng of fragments with about 100 bp were labeled with adaptors comprising a random 6 bp identifier. There are about 4.6×10^{12} double-stranded fragments in this sample, while the number of unique identifier is 4^6 or 4096. Therefore, there are not enough adapters to provide each of the fragments with a unique identifier. Even a 10 bp random identifier would have 4^{10} or 2.7×10^8 unique molecules, about 10,000-fold lower than necessary.

In Example 5 Applicant describes labeling 100 ng of 200 bp fragments with an adapter comprising a 6 bp random identifier. The number of double-stranded fragments would be 4.6×10^{11} , and the number of unique adapters 4096. In conclusion, Applicant did not describe any adapters which provide identifiers capable of uniquely labeling every fragment in a sample.

As a consequence, the priority date of the instant claims is the filing date of the instant application, July 21, 2021.

Information Disclosure Statement

4. The information disclosure statement (IDS) submitted on August 4, 2022 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

5. No references were found teaching or suggesting claims 22-37. The closest prior art reference, Doan et al. (US 2022/0145380 A1; filed November 2019) teaches detection of mutations in sequenced DNA samples by appending adapters with unique identifiers to DNA fragments, grouping the fragments using the unique identifiers and removing duplicate reads, but does not teach or suggest using a combination of target sequence and identifier sequence for duplicate removal.

Further, as can be seen from Kinde et al. (PNAS, vol. 108, pp. 9530-9535, 2011), unique fragment identifiers were used to create consensus sequences from many sequence read duplicates, and the duplicates were not removed.

As a consequence, claims 22-37 are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached M-F 9-7.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available

Art Unit: 1637

to registered users. To file and manage patent submissions in Patent Center, visit:

<https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/TERESA E STRZELECKA/
Primary Examiner, Art Unit 1637
March 17, 2023

<i>Notice of References Cited</i>	Application/Control No. 17/381,888		Applicant(s)/Patent Under Reexamination Amorese et al.	
	Examiner TERESA E STRZELECKA		Art Unit 1637	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20220145380-A1	05-2022	DOAN; Ryan N.	C12Q1/6851	1/1
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Kinde, I. et al., Detection and quantification of rare mutations with massively parallel sequencing, PNAS, vol. 108, pp. 9530-9535, 2011) (Year: 2011)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes 	Application/Control No. 17/381,888	Applicant(s)/Patent Under Reexamination Amorese et al.
	Examiner TERESA E STRZELECKA	Art Unit 1637

CPC - Searched*		
Symbol	Date	Examiner
(C12Q1/6869 OR C12Q2525/191 OR C12Q2525/179) plus text search	03/15/2023	/TS/

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Inventor name search	03/14/2023	/TS/
Google	03/15/2023	/TS/
IDS (8/4/22)	03/15/2023	/TS/
PE2E search	03/15/2023	/TS/
PE2E search	03/16/2023	/TS/

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	(C12Q1/6869 OR C12Q2525/191 OR C12Q2525/179) plus text search	03/15/2023	/TS/

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<i>Search Notes</i> 	Application/Control No. 17/381,888	Applicant(s)/Patent Under Reexamination Amorese et al.
	Examiner TERESA E STRZELECKA	Art Unit 1637

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Issue Classification 	Application/Control No. 17/381,888	Applicant(s)/Patent Under Reexamination Amorese et al.	
	Examiner TERESA E STRZELECKA	Art Unit 1637	

CPC						
Symbol					Type	Version
C12Q	/	1	/	6874	F	2013-01-01
C12Q	/	1	/	6869	I	2013-01-01
C12Q	/	2525	/	179	A	2013-01-01
C12Q	/	2525	/	191	A	2013-01-01

CPC Combination Sets								
Symbol					Type	Set	Ranking	Version
C12Q	/	1	/	6869	I	1	1	2013-01-01
C12Q	/	2525	/	179	I	1	2	2013-01-01
C12Q	/	2525	/	191	I	1	3	2013-01-01
C12Q	/	2537	/	157	I	1	4	2013-01-01
C12Q	/	2563	/	179	I	1	5	2013-01-01
C12Q	/	2565	/	514	I	1	6	2013-01-01
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C12Q	/	2537	/	159	I	2	6	2013-01-01
C12Q	/	2563	/	179	I	2	7	2013-01-01

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	16	
/TERESA E STRZELECKA/ Primary Examiner, Art Unit 1637	17 March 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	22	None

Issue Classification 	Application/Control No. 17/381,888	Applicant(s)/Patent Under Reexamination Amorese et al.	
	Examiner TERESA E STRZELECKA	Art Unit 1637	

INTERNATIONAL CLASSIFICATION			
CLAIMED			
C12Q1/6874	/	1	/ 6874
C12Q1/6869	/	1	/ 6869

NON-CLAIMED			
/		/	

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	16	
/TERESA E STRZELECKA/ Primary Examiner, Art Unit 1637	17 March 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	22	None

<i>Issue Classification</i> 	Application/Control No. 17/381,888	Applicant(s)/Patent Under Reexamination Amorese et al.
	Examiner TERESA E STRZELECKA	Art Unit 1637

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47															
CLAIMS															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 16	
/TERESA E STRZELECKA/ Primary Examiner, Art Unit 1637 (Primary Examiner) _____ (Date) 17 March 2023		O.G. Print Claim(s) 22	O.G. Print Figure None

Bibliographic Data

Application No: 17/381,888

Foreign Priority claimed: ☐ Yes ☒ No

35 USC 119 (a-d) conditions met: ☐ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged: /TERESA E STRZELECKA/

Examiner's Signature

Initials

Title:

COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A
DUPLICATE SEQUENCING READ

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
07/21/2021	506	1637	NUGN-001/04US 34079/550
RULE			

APPLICANTS

Tecan Genomics, Inc., Redwood City, CA,

INVENTORS

Douglas A. Amorese, Los Altos, CA, UNITED STATES

Jonathan Scolnick, San Francisco, CA, UNITED STATES

Ben Schroeder, San Mateo, CA, UNITED STATES

CONTINUING DATA

This application is a CON of 16743604 01/15/2020 PAT 11098357

16743604 is a CON of 15406002 01/13/2017 PAT 10570448

15406002 is a DIV of 14540917 11/13/2014 PAT 9546399

14540917 has PRO of 61903826 11/13/2013

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

08/10/2021

STATE OR COUNTRY

UNITED STATES

ADDRESS

BROWN RUDNICK LLP

ONE FINANCIAL CENTER

BOSTON, MA 02111

UNITED STATES

FILING FEE RECEIVED

\$1,820

Doc code: IDS

PTO/SB/08a (01-22)

Doc description: Information Disclosure Statement (IDS) Filed

Approved for use through 05/31/2024. 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		17381888
	Filing Date		2021-07-21
	First Named Inventor	Douglas A. Amorese	
	Art Unit	N/A	
	Examiner Name	Not Yet Assigned Teresa Strzelecka	
	Attorney Docket Number	NUGN-001/04US 34079/550	

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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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	2	20090036663	A1	2009-02-05	Kurn	
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**INFORMATION DISCLOSURE
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Examiner Name	Not Yet Assigned	Teresa Strzelecka
Attorney Docket Number	NUGN-001/04US 34079/550	

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Attorney Docket Number	NUGN-001/04US 34079/550	

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Attorney Docket Number	NUGN-001/04US 34079/550	

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Examiner Name	Not Yet Assigned	Teresa Strzelecka
Attorney Docket Number	NUGN-001/04US 34079/550	

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Art Unit	N/A	
Examiner Name	Not Yet Assigned	Teresa Strzelecka
Attorney Docket Number	NUGN-001/04US 34079/550	

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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
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PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L9	34	unique near3 (barcod\$6 OR index\$76 OR tag\$7 OR identifier) AND duplicate near3 read AND read near3 (group\$8 OR famil\$8) near3 (barcod\$6 OR index\$76 OR tag\$7 OR identifier) AND duplicate near3 (remov\$7 OR collaps\$7)	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/03/16 05:14 PM
L10	42	unique near3 (barcod\$6 OR index\$76 OR tag\$7 OR identifier) AND duplicate near3 read AND read near3 (group\$8 OR famil\$8) near3 (barcod\$6 OR index\$76 OR tag\$7 OR identifier) AND duplicate near3 (remov\$7 OR collaps\$7)	(FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); EPO; JPO)	ADJ	ON	ON	2023/03/16 05:44 PM
L11	5	"63039174".fmid.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; JPO)	ADJ	ON	ON	2023/03/16 05:52 PM
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There are no Interference searches to show.

Doc code: IDS

PTO/SB/08a (01-22)

Doc description: Information Disclosure Statement (IDS) Filed

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	Examiner Name	Not Yet Assigned Teresa Strzelecka	
	Attorney Docket Number	NUGN-001/04US 34079/550	

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First Named Inventor	Douglas A. Amorese
Art Unit	N/A
Examiner Name	Not Yet Assigned Teresa Strzelecka
Attorney Docket Number	NUGN-001/04US 34079/550

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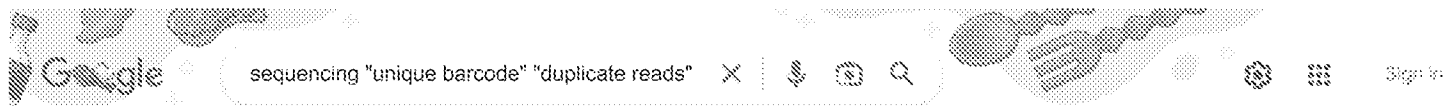
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Wiley
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The University of Texas at Austin
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Alevin: An integrated method for dscRNA-seq quantification

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10x Genomics

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by JM Yost · 2020 · Cited by 20 — ... first ligated to unique barcode sequences (red, blue, and green). ... UMI-based error correction leverages duplicate reads to determine ...

 **GitHub**
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Samples are assigned unique barcode tracking numbers and have a detailed sample ... Duplicate reads, QC-failed reads, supplementary reads, secondary reads ...



Journal of Thoracic Oncology

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A Clinician's Guide to Bioinformatics for Next-Generation ...

by NB Larson · Cited by 1 — ... together for sequencing in a single run using unique barcode labels. ... First, duplicate reads (i.e., reads originated from the same ...



Rockefeller University Press

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Nov 16, 2021 — ... plate received a unique barcode downstream of the T7 promoter. ... T7 duplicate reads were filtered based on read 1 start positions ...



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Barcoded bulk QTL mapping reveals highly polygenic and ...

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Labome

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American Society for Microbiology

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A Universal Next-Generation Sequencing Protocol To ...

by LA Moser · 2016 · Cited by 32 — ... a primer with a random hexamer coupled to a unique barcode (BC-N6). ... Duplicate reads were removed, reads were demultiplexed based on ...



HackMD

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Reference-guided RAD analysis workflow - HackMD

During library prep we use a unique barcode to identify each sample in each well, ... percent of reads mapping, and proportion of duplicate reads.



OpenReview

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Machine learning for discovery: deciphering RNA splicing logic

by SE Liao — Each reporter includes a unique barcode at the end of the third exon so that ... unique molecular identifiers (UMIs), the fraction of duplicate reads in ...



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Notes on RNA-seq – Logan Thrasher Collins - WordPress.com

containing barcoded primers (each droplet possesses a unique barcode) with poly-dT sequences ... underrepresented), duplicate reads, and other artifacts.



Thermo Fisher Scientific

<https://assets.thermofisher.com/LSG/manuals/PDF>

Torrent Suite™ Software 5.12 - USER GUIDE

Nov 5, 2019 — Assign a unique barcode to each sample in a Sample Set. ... Marking duplicate reads is not appropriate for Ion AmpliSeq™ data, because many.



EMBO Press

<https://www.embopress.org/doi/msh.20178046>**Using single-cell genomics to understand developmental ...**

by JA Griffiths · 2018 · Cited by 177 — ... randomly generated nucleotide sequences that allow PCR duplicate reads to ... sequences of fluorescence across washes, building up a unique...



ugent.be

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by AR Vierstraete · 2022 · Cited by 1 — which contains the unique barcode- primer sequences, a fastq file with the sequences, and the ... duplicate reads in the comparison.



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rug.nl

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by H van den Bos · 2016 · Cited by 143 — Illumina sequencing. Since each single-cell library received a unique barcode, ... (version 0.1.18) [25] and duplicate reads were marked.



AHA Journals

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Washington University in St. Louis

<https://digitalcommons.wustl.edu/viewcontent/PDF>**Sequencing-based measurable residual disease testing in ...**

by JM Yoeast · 2020 · Cited by 20 — distinguish duplicate reads originating from the same DNA ... are first ligated to unique barcode sequences (red, blue, and green).



EpiCypher

<https://www.epicypher.com/content/manuals/PDF>**14-1001-2-cut-and-run-library-prep-kit-manual.pdf - EpiCypher**

Each primer incorporates a unique barcode ... remove adapter dimers (using gel purification, FAQ 3), filter out duplicate reads, and/or increase sequencing ...



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EFS ID:	46340787
Application Number:	17381888
International Application Number:	
Confirmation Number:	6188
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ
First Named Inventor/Applicant Name:	Douglas A. Amorese
Customer Number:	21710
Filer:	Thomas C. Meyers/Kelley Warren
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	NUGN-001/04US 34079/550
Receipt Date:	04-AUG-2022
Filing Date:	21-JUL-2021
Time Stamp:	21:53:16
Application Type:	Utility under 35 USC 111(a)

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EFS ID:	46340839
Application Number:	17381888
International Application Number:	
Confirmation Number:	6188
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ
First Named Inventor/Applicant Name:	Douglas A. Amorese
Customer Number:	21710
Filer:	Thomas C. Meyers/Kelley Warren
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	NUGN-001/04US 34079/550
Receipt Date:	04-AUG-2022
Filing Date:	21-JUL-2021
Time Stamp:	21:55:11
Application Type:	Utility under 35 USC 111(a)

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EFS ID:	46340587
Application Number:	17381888
International Application Number:	
Confirmation Number:	6188
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ
First Named Inventor/Applicant Name:	Douglas A. Amorese
Customer Number:	21710
Filer:	Thomas C. Meyers/Kelley Warren
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	NUGN-001/04US 34079/550
Receipt Date:	04-AUG-2022
Filing Date:	21-JUL-2021
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Application Type:	Utility under 35 USC 111(a)

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	17381888
	Filing Date	2021-07-21
	First Named Inventor	Douglas A. Amorese
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	NUGN-001/04US 34079/550

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	2	2013/191775	WO	A2	2013-12-27	Nugen Technologies, Inc		
	3	2014/039556	WO	A1	2014-03-13	Guardant Health, Inc		

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number		17381888
Filing Date		2021-07-21
First Named Inventor	Douglas A. Amorese	
Art Unit	N/A	
Examiner Name	Not Yet Assigned	
Attorney Docket Number	NUGN-001/04US 34079/550	

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6	2014/150931	WO	A1	2014-09-25	Nugen Technologies, Inc		
7	2015/031691	WO	A1	2015-03-05	Cellular Research, Inc		
8	2015/073711	WO	A1	2015-05-21	Nugen Technologies, Inc		
9	2015/131107	WO	A1	2015-09-03	Nugen Technologies, Inc		
10	89/09284	WO	A1	1989-10-05	Univ Iowa Res Found		
11	92/07951	WO	A1	1992-05-14	Kow, Yoke		
12	93/18052	WO	A1	1993-09-16	Isis Pharmaceuticals, Inc		
13	94/16090	WO	A1	1994-07-21	Molecular Tool, Inc		
14	96/40998	WO	A1	1996-12-19	Pioneer Hi-bred International, Inc		

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

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Filing Date	2021-07-21
First Named Inventor	Douglas A. Amorese
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	NUGN-001/04US 34079/550

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20	99/10540	WO	A1	1999-03-04	Lopez, Osvaldo		
21	99/11819	WO	A1	1999-03-11	Perkin Elmer Corp		
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Application Number	17381888
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Art Unit	N/A
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Application Number	17381888
Filing Date	2021-07-21
First Named Inventor	Douglas A. Amorese
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	NUGN-001/04US 34079/550

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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	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2022-08-04
Name/Print	Thomas C. Meyers	Registration Number	36,989

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EFS ID:	46340448
Application Number:	17381888
International Application Number:	
Confirmation Number:	6188
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ
First Named Inventor/Applicant Name:	Douglas A. Amorese
Customer Number:	21710
Filer:	Thomas C. Meyers/Kelley Warren
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	NUGN-001/04US 34079/550
Receipt Date:	04-AUG-2022
Filing Date:	21-JUL-2021
Time Stamp:	21:44:54
Application Type:	Utility under 35 USC 111(a)

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38	Foreign Reference	WO0228876A2.pdf	3715353	no	97
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39	Foreign Reference	WO0229117A2.pdf	4384957	no	82
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41	Foreign Reference	WO0248402A2.pdf	9001783	no	172
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42	Foreign Reference	WO2003004690.pdf	3805408	no	77
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43	Foreign Reference	WO03027259A2.pdf	2369559	no	49
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44	Foreign Reference	WO03078645A2.pdf	4814705	no	81
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Warnings:					
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45	Foreign Reference	WO03083435A2.pdf	5893654	no	114
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46	Foreign Reference	WO03106642A2.pdf	3399953	no	71
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47	Foreign Reference	WO2004011665A2.pdf	6663836	no	114
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48	Foreign Reference	WO0009756A1.pdf	2439950	no	49
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49	Foreign Reference	WO0055364A2.pdf	5236658	no	168
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50	Foreign Reference	WO03002736A2.pdf	3507273	no	66
			41087e94b669a728739e892267589ebd8a02c6b8		
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51	Foreign Reference	WO03012118A1.pdf	1899370	no	39
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52	Foreign Reference	WO2004070007.pdf	3695746	no	73
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53	Foreign Reference	WO2004092418A2.pdf	6086132	no	96
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54	Foreign Reference	WO2005003375A2.PDF	11674420	no	200
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55	Foreign Reference	WO2005038427.pdf	1599606	no	51
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56	Foreign Reference	WO2005065321A2.pdf	5854379	no	95
			53ba149f09d55c3aeab73560f962d05462130ac6		
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57	Foreign Reference	WO2006081222A2.pdf	2587188 d535e68c6ec955c8c6b8adc5cf662afc2438f e2c	no	40
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58	Foreign Reference	WO2006086668A2.pdf	6034034 377d7437715e4f7a131446b84c65235b424 b9b43	no	98
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59	Foreign Reference	WO2006137733.pdf	3359055 ef603385fe8e2c637ff28cceb77c28711606b fee	no	75
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60	Foreign Reference	WO2007018601A1.pdf	19862831 89cbd0a94dc6b7893bc60a3c1136805fb5e 027fe	no	152
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Total Files Size (in bytes):			228626292		
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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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	2	4458066		1984-07-03	Caruthers et al.	
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21	5216141		1993-06-01	Benner	
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98	6174680		2001-01-16	Makrigiorgos	
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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	17381888
Filing Date	2021-07-21
First Named Inventor	Douglas A. Amorese
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	NUGN-001/04US 34079/550

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	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2022-08-04
Name/Print	Thomas C. Meyers	Registration Number	36,989

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	20090029385	A1	2009-01-29	Christians et al.	
	2	20090036663	A1	2009-02-05	Kurn	
	3	20090061425	A1	2009-03-05	Lo et al.	
	4	20090061439	A1	2009-03-05	Buzby	
	5	20090068645	A1	2009-03-12	Sibson	

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6	20090068655	A1	2009-03-12	Williams	
7	20090068709	A1	2009-03-12	Kurn et al.	
8	20090105081	A1	2009-04-23	Rodesch et al.	
9	20090117573	A1	2009-05-07	Fu et al.	
10	20090117621	A1	2009-05-07	Boutell et al.	
11	20090124514	A1	2009-05-14	Fu et al.	
12	20090127589	A1	2009-05-21	Rothberg et al.	
13	20090130721	A1	2009-05-21	Kurn et al.	
14	20090203085	A1	2009-08-13	KURN et al.	
15	20090203531	A1	2009-08-13	Kurn	
16	20090233802	A1	2009-09-17	Bignell et al.	

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17	20090233804	A1	2009-09-17	Kurn et al.	
18	20090239232	A1	2009-09-24	Kurn	
19	20090275486	A1	2009-11-05	Kurn et al.	
20	20090280538	A1	2009-11-12	Patel et al.	
21	20090298075	A1	2009-12-03	Travers et al.	
22	20100015666	A1	2010-01-21	Brenner et al.	
23	20100021973	A1	2010-01-28	Makarov et al.	
24	20100022403	A1	2010-01-28	Kurn et al.	
25	20100029511	A1	2010-02-04	Raymond et al.	
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27	20100113296	A1	2010-05-06	Myerson	

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28	20100129879	A1	2010-05-27	Ach et al.	
29	20100137143	A1	2010-06-03	Rothberg et al.	
30	20100159559	A1	2010-06-24	Kurn et al.	
31	20100167954	A1	2010-07-01	Earnshaw et al.	
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33	20100203597	A1	2010-08-12	Chen et al.	
34	20100267043	A1	2010-10-21	Braverman et al.	
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36	20100311066	A1	2010-12-09	Kurn	
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39	20110039732	A1	2011-02-17	Raymond et al.	
40	20110104785	A1	2011-05-05	Vaidyanathan et al.	
41	20110105364	A1	2011-05-05	Kurn	
42	20110129827	A1	2011-06-02	Causey et al.	
43	20110189679	A1	2011-08-04	Kurn et al.	
44	20110224105	A1	2011-09-15	Kurn et al.	
45	20110269647	A1	2011-11-03	ULE et al.	
46	20110288780	A1	2011-11-24	Rabinowitz et al.	
47	20110294132	A1	2011-12-01	Kurn	
48	20110319290	A1	2011-12-29	Raymond et al.	
49	20120003657	A1	2012-01-05	Mylykangas et al.	

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50	20120028310	A1	2012-02-02	Kurn et al.	
51	20120045797	A1	2012-02-23	Kurn et al.	
52	20120071331	A1	2012-03-22	Casbon et al.	
53	20120074925	A1	2012-03-29	Oliver	
54	20120102054	A1	2012-04-26	POPESCU et al.	
55	20120107811	A1	2012-05-03	Kelso et al.	
56	20120122701	A1	2012-05-17	Ryan et al.	
57	20120149068	A1	2012-06-14	Kurn	
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60	20120190587	A1	2012-07-26	Kurn et al.	

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61	20120208705	A1	2012-08-16	Steemers et al.	
62	20120220483	A1	2012-08-30	KURN et al.	
63	20120220494	A1	2012-08-30	Samuels et al.	
64	20120237943	A1	2012-09-20	Soldatov et al.	
65	20120238738	A1	2012-09-20	Hendrickson	
66	20120245041	A1	2012-09-27	Brenner et al.	
67	20120270212	A1	2012-10-25	RABINOWITZ et al.	
68	20120283145	A1	2012-11-08	Wang	
69	20120289426	A1	2012-11-15	Roos et al.	
70	20120309002	A1	2012-12-06	Link	
71	20130005585	A1	2013-01-03	Anderson et al.	

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72	20130059738	A1	2013-03-07	LEAMON et al.	
73	20130231253	A1	2013-09-05	Amorese et al.	
74	20140038188	A1	2014-02-06	Kurn	
75	20140038236	A1	2014-02-06	Kurn et al.	
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78	20140274731	A1	2014-09-18	RAYMOND et al.	
79	20140274738	A1	2014-09-18	Amorese et al.	
80	20140303000	A1	2014-10-09	Armour	
81	20140378345	A1	2014-12-25	Hindson et al.	
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83	20150017635	A1	2015-01-15	Mylykangas et al.	
84	20150101595	A1	2015-04-16	Hancock et al.	
85	20150133319	A1	2015-05-14	Fu et al.	
86	20150284769	A1	2015-10-08	Schroeder	
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90	20160122756	A1	2016-05-05	Armour	
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94	20160220994	A1	2016-08-04	Wright
95	20160251711	A1	2016-09-01	AMORESE et al.
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97	20160275240	A1	2016-09-22	HUELGA et al.
98	20160296930	A1	2016-10-13	Matear et al.

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	1	2006/086668	WO	A2	2006-08-17	Epict Technologies		
	2	2006/137733	WO	A1	2006-12-28	Keygene NV		
	3	2007/018601	WO	A1	2007-02-15	Rubicon Genomics, Inc		
	4	2007/019444	WO	A2	2007-02-15	Euclid Diagnostics LLC		

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5	2007/030759	WO	A2	2007-03-15	Nugen Technologies, Inc		
6	2007/037678	WO	A2	2007-04-05	Keygene NV		
7	2007/052006	WO	A1	2007-05-10	Solexa Ltd		
8	2007/057652	WO	A1	2007-05-24	Solexa Ltd		
9	2007/073165	WO	A1	2007-06-28	Keygene NV		
10	2007/136717	WO	A1	2007-11-29	Nugen Technologies, Inc		
11	2008/005459	WO	A2	2008-01-10	Nugen Technologies, Inc		
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13	2008/033442	WO	A2	2008-03-20	Univ Leland Stanford Junior		
14	2008/093098	WO	A2	2008-08-07	Solexa Ltd		
15	2008/115185	WO	A2	2008-09-25	Nimblegen Systems, Inc		

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17	2009/053039	WO	A1	2009-04-30	Roche Diagnostics GmbH		
18	2009/102878	WO	A2	2009-08-20	Nugen Technologies, Inc		
19	2009/102896	WO	A2	2009-08-20	Nugen Technologies, Inc		
20	2009/112844	WO	A1	2009-09-17	Stichting Het NI Kanker I		
21	2009/117698	WO	A2	2009-09-24	Nugen Technologies, Inc		
22	2009/120372	WO	A2	2009-10-01	Pacific Biosciences Of California, Inc		
23	2009/120374	WO	A2	2009-10-01	Pacific Biosciences Of California, Inc		
24	2010/003153	WO	A2	2010-01-07	Life Technologies Corp		
25	2010/030683	WO	A1	2010-03-18	Rosetta Inpharmatics LLC		
26	2010/039991	WO	A2	2010-04-08	Texas A & M Univ Sys		

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27	2010/063711	WO	A1	2010-06-10	Angeletti P Ist Recherche Bio		
28	2010/064893	WO	A1	2010-06-10	Keygene NV		
29	2010/085715	WO	A1	2010-07-29	Quanta Biosciences		
30	2010/091246	WO	A2	2010-08-12	Univ Northwestern		
31	2010/115154	WO	A1	2010-10-07	Fluidigm Corp		
32	2010/129937	WO	A2	2010-11-11	Life Technologies Corp		
33	2011/003630	WO	A1	2011-01-13	Max Planck Gesellschaft		
34	2011/009941	WO	A1	2011-01-27	Olink Genomics AB		
35	2011/019964	WO	A1	2011-02-17	Nugen Technologies, Inc		
36	2011/032053	WO	A1	2011-03-17	Nugen Technologies, Inc.		
37	2011/053987	WO	A1	2011-05-05	Nugen Technologies, Inc		

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38	2011/151777	WO	A1	2011-12-08	Consiglio Nazionale Ricerche		
39	2011/156529	WO	A2	2011-12-15	Nugen Technologies, Inc		
40	2011032040	WO	A1	2011-03-17	Centrillion Technology Holding Corp		
41	2012/013932	WO	A1	2012-02-02	Univ St Andrews		
42	2012/054873	WO	A3	2012-08-23	Cold Spring Harbor Lab		
43	2012/061832	WO	A1	2012-05-10	Illumina, Inc		
44	2012/103154	WO	A1	2012-08-02	Nugen Technologies, Inc		
45	2013/059740	WO	A1	2013-04-25	Foundation Medicine, Inc		
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47	2013/112923	WO	A1	2013-08-01	Nugen Technologies, Inc		
48	2013/130512	WO	A3	2013-10-24	Univ North Carolina		

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49	2013/138510	WO	A9	2014-07-10	Patel, Abhijit Ajit		
50	2013/177220	WO	A1	2013-11-28	Scripps Research Inst		

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	1	Wolford, 2000, High-throughput SNP detection by using DNA pooling and denaturing high performance liquid chromatography (DHPLC), Hum Genet 107:483-487	
	2	Xi, 2011, Copy number variation detection in whole-genome sequencing data using the Bayesian information criterion, PNAS 108(46):e1128-e1136	
	3	Xu, 2012, FastUniq: A fast de novo duplicates removal tool for paired short reads, PLoSOne 7(12):e52249	

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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	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2022-08-04
Name/Print	Thomas C. Meyers	Registration Number	36,989

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 records.
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.



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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
17/381,888	07/21/2021	Douglas A. Amorese	NUGN-001/04US 34079/550

CONFIRMATION NO. 6188

POA ACCEPTANCE LETTER

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111



Date Mailed: 12/22/2021

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 12/17/2021.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

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.

/agizaw/

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

☒ Practitioners associated with Customer Number:

21710

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

☐ The address associated with Customer Number:

OR

☐ Firm or individual name		
Address		
City	State	Zip
Country		
Telephone	Email	

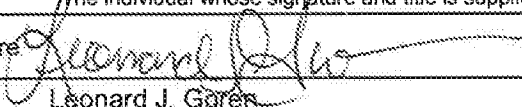
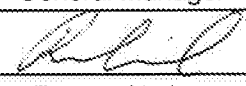
Assignee name and address:

Tecan Genomics, Inc.
900 Chesapeake Drive
Redwood City, California 94063

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of the practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee.

Signature 	Date 6 December 2021
Name Leonard J. Goren	Telephone 650-590-3660
Title General Manager	
Signature 	Date 6 December 2021
Name Rasmus Lind	Telephone
Title Vice President Global Sales	

Electronic Acknowledgement Receipt

EFS ID:	44546595
Application Number:	17381888
International Application Number:	
Confirmation Number:	6188
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ
First Named Inventor/Applicant Name:	Douglas A. Amorese
Customer Number:	21710
Filer:	Thomas C. Meyers/Sandra Richards
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	NUGN-001/04US 34079/550
Receipt Date:	17-DEC-2021
Filing Date:	21-JUL-2021
Time Stamp:	12:50:45
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Assignee showing of ownership per 37 CFR 3.73	NUGN-001-01US-373-statement.pdf	25972	no	2
			bd3d403fb79184b5fa9b781f92bd09c459b54347		

Warnings:

QIAGEN Exhibit 1012.0192

Information:					
2	Power of Attorney	Tecan-POA-120621.pdf	83539	no	1
			d84541658fd7a3f8276760e65ab3d5eee2f29a76		
Warnings:					
Information:					
			Total Files Size (in bytes):		
			109511		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Douglas A. Amorese et al.

Application No./Patent No.: 17/381,888 Filed/Issue Date: July 21, 2021

Titled: COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ

Tecan Genomics, Inc., a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☒ The assignee of the entire right, title, and interest.
2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):
- ☐ The extent (by percentage) of its ownership interest is _____ %. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.
- ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (e.g., bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

- A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.
- B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Douglas A. Amorese, et al. To: Nugen Technologies, Inc.
The document was recorded in the United States Patent and Trademark Office at
Reel 053605, Frame 0123, or for which a copy thereof is attached.
2. From: Nugen Technologies, Inc. To: Tecan Genomics, Inc.
The document was recorded in the United States Patent and Trademark Office at
Reel 056918, Frame 0037, or for which a copy thereof is attached.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Signature /Thomas C. Meyers/_____
Date December 17, 2021_____
Printed or Typed Name Thomas C. Meyers_____
Title or Registration Number 36,989



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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
17/381,888	07/21/2021	Douglas A. Amorese	NUGN-001/04US 34079/550

CONFIRMATION NO. 6188

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PUBLICATION NOTICE



OC000000129893290

Title:COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ

Publication No.US-2021-0355537-A1

Publication Date:11/18/2021

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently <http://www.uspto.gov/patft/>.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently <https://portal.uspto.gov/pair/PublicPair>. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 17/381,888				
APPLICATION AS FILED - PART I										
(Column 1)		(Column 2)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY				
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)			
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	320			
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A			N/A	700			
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	800			
TOTAL CLAIMS (37 CFR 1.16(j))	16	minus 20 = *			OR	x 100 =	0.00			
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1	minus 3 = *				x 480 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL		TOTAL	1820			
APPLICATION AS AMENDED - PART II										
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY		
AMENDMENT A		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x	=	OR	x	=
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x	=	OR	x	=
	Application Size Fee (37 CFR 1.16(s))							OR		
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	
AMENDMENT B		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x	=	OR	x	=
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x	=	OR	x	=
	Application Size Fee (37 CFR 1.16(s))							OR		
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.										



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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
17/381,888	07/21/2021		1820	NUGN-001/04US 34079/550	16	1

CONFIRMATION NO. 6188

FILING RECEIPT



0000000127621288

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

Date Mailed: 08/11/2021

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)

Douglas A. Amorese, Los Altos, CA;
Jonathan Scolnick, San Francisco, CA;
Ben Schroeder, San Mateo, CA;

Applicant(s)

Tecan Genomics, Inc., Redwood City, CA;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 16/743,604 01/15/2020 PAT 11098357
which is a CON of 15/406,002 01/13/2017 PAT 10570448
which is a DIV of 14/540,917 11/13/2014 PAT 9546399
which claims benefit of 61/903,826 11/13/2013

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

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: 08/10/2021

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 17/381,888**

Projected Publication Date: 11/18/2021

Non-Publication Request: No

Early Publication Request: No

Title

COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ

Preliminary Class

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).

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Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

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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 Assets Control, 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.



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Office of the Chief Financial Officer

Document Code:WFEE

User :C43106

Sale Adjustment Accounting Date:08/10/2021

Effective Date	Sale Accounting Date	Sale Item Reference Number
07/21/2021	08/10/2021	17381888

Document Number	Fee Code	Fee Code Description	Amount Paid	Payment Method
I202180D59200876	1202	CLAIMS IN EXCESS OF 20	\$100.00	DA



United States Patent and Trademark Office

Office of the Chief Financial Officer

Document Code:WFEE

User :C43106

Refund Accounting Date:08/10/2021

Effective Date	Sale Item Reference Number	Refund Total
07/21/2021	17381888	\$100.00

Document Number	Fee Code	Fee Code Description	Amount Paid	Payment Method	Account Number
I202180D59200876	1202	CLAIMS IN EXCESS OF 20	\$100.00	DA	500369

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Tecan Genomics, Inc.	ART UNIT:	Not Yet Assigned
SERIAL NUMBER:	17/381,888	CONF. NO.:	6188
FILING DATE:	July 21, 2021	EXAMINER:	Not Yet Assigned
TITLE: COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ			

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

FILED ELECTRONICALLY

PRELIMINARY AMENDMENT

Prior to examination of the above U.S. nonprovisional patent application, please amend the claims and consider the following remarks. Applicant believes that no fees are due in connection with this Preliminary Amendment. However, Applicant authorizes any required fees to be charged to Deposit Account No. 500369.

Amendments to the claims begin on page 2.

Remarks begin on page 5.

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

1 – 21 (Cancelled).

22. (New) A method for detecting duplicate sequencing reads, the method comprising:
obtaining amplicons each comprising an amplified fragment of a nucleic acid with an appended adaptor, wherein each adaptor comprises an identifier site comprising a plurality of nucleotides unique to the amplified fragment;

sequencing the amplicons to generate sequence reads that include identifier and target sequences; and

identifying sequence reads with identical identifier and target sequences as duplicates.

23. (New) The method of claim 22, further comprising removing a duplicate read from the sequence reads.

24. (New) The method of claim 22, wherein the identifier site is sequenced with the target sequence.

25. (New) The method of claim 22, wherein the identifier site is sequenced separately from the target sequence.

26. (New) The method of claim 22, wherein the amplicons include amplified fragments of cDNA.

27. (New) The method of claim 22, wherein the amplicons include amplified fragments of genomic DNA.
28. (New) The method of claim 22, wherein each adaptor includes a primer binding site.
29. (New) The method of claim 28, wherein the primer binding site is a universal target sequencing primer binding site.
30. (New) The method of claim 22, wherein each unique identifier is at least 6 nucleotides in length.
31. (New) The method of claim 22, wherein the adaptors have been appended to the fragments using a ligation reaction or a priming reaction.
32. (New) The method of claim 22, wherein each unique identifier is at a junction between the adapter and the nucleic fragment.
33. (New) The method of claim 22, further comprising determining the genomic start position for each sequencing read.
34. (New) The method of claim 22, wherein each adaptor further comprises an indexing site.
35. (New) The method of claim 22, wherein each identifier site is at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length.

36. (New) The method of claim 22, further comprising obtaining a sample comprising the nucleic acid, fragmenting the nucleic acid to yield the fragments of nucleic acid, appending the adaptors to the fragments of nucleic acid, and performing an amplification reaction to obtain the amplicons.

37. (New) The method of claim 22, wherein the amplicons include amplified fragment of a nucleic acid from a first sample with an appended adaptor that includes a first index and amplified fragment of a nucleic acid from a second sample with an appended adaptor that includes a second index, and the method includes separating sequence reads by sample based on the first or second index.

REMARKS

By this Preliminary Amendment, Applicant has herein cancelled claims 1-21 and added new claims 22-37. Accordingly, upon entry of these claim amendments, claims 22-37 will be pending, of which claim 22 is the sole independent claim.

Support for new claims 22-37 can be found throughout the originally filed application, including at paragraph [0047] on page 17; paragraph [0064] on page 25; paragraph [0065] on pages 25-26; paragraph [0030] on page 8; paragraph [0029] on page 7; and paragraph [0069] on page 27.

If there are any questions regarding this application, the Office is invited and encouraged to contact Applicant's representatives at the telephone number provided.

Dated: July 28, 2021

Respectfully submitted,
BROWN RUDNICK LLP

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: 617-856-8483

/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

Electronic Acknowledgement Receipt

EFS ID:	43365410
Application Number:	17381888
International Application Number:	
Confirmation Number:	6188
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ
First Named Inventor/Applicant Name:	Douglas A. Amorese
Customer Number:	21710
Filer:	Thomas C. Meyers/Sandra Richards
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	NUGN-001/04US 34079/550
Receipt Date:	28-JUL-2021
Filing Date:	
Time Stamp:	15:27:17
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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Multipart Description/PDF files in .zip description			
Document Description	Start	End	
Preliminary Amendment	1	1	
Claims	2	4	
Applicant Arguments/Remarks Made in an Amendment	5	5	

Warnings:

Information:

Total Files Size (in bytes):	125699
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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

=====

Sequence Listing was accepted.

If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).

Reviewer: Anjum, Durreshwar

Timestamp: [year=2021; month=7; day=22; hr=8; min=35; sec=35; ms=227;]

=====

Application No: 17381888 Version No: 1.0

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Output Set:

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Finished: 2021-07-21 16:14:55.585
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Total Errors: 0
No. of SeqIDs Defined: 9
Actual SeqID Count: 9

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Input Set:

Output Set:

Started: 2021-07-21 16:14:54.817
Finished: 2021-07-21 16:14:55.585
Elapsed: 0 hr(s) 0 min(s) 0 sec(s) 768 ms
Total Warnings: 47
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Actual SeqID Count: 9

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Tecan Genomics, Inc.	ART UNIT:	Not Yet Assigned
SERIAL NUMBER:	Continuation of US Serial No. 16/743,604	CONF. NO.:	Not Yet Assigned
FILING DATE:	Herewith	EXAMINER:	Not Yet Assigned
TITLE:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

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PRELIMINARY AMENDMENT

Prior to examination of the above U.S. nonprovisional patent application, please amend the claims and the specification and consider the following remarks. Applicants believe that no fees are due with respect to this Preliminary Amendment.

Amendments to the specification begin on page 2.

A **Sequence Listing** is referenced on page 3.

Remarks begin on page 3.

Applicant believes that no fees are due in connection with this Preliminary Amendment. However, Applicant authorizes any required fees to be charged to Deposit Account No. 500369.

AMENDMENTS TO THE SPECIFICATION

Please amend the “CROSS-REFERENCE” section on page 1, paragraph [0001] of the as-filed specification as follows:

CROSS-REFERENCE

This application is a continuation of U.S. Application Serial No. 16/743,604, filed January 15, 2020, which is a continuation of U.S. Application Serial No. 15/406,002, filed January 13, 2017, which is a divisional of U.S. Serial No. 14/540,917, filed November 13, 2014, which claims the benefit of U.S. Provisional Application No. 61/903,826, filed November 13, 2013, which [[is]] are each herein incorporated by reference in [[its]] their entirety.

Please amend the “DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY” section on page 1, paragraph [0002] of the as-filed specification as follows:

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: NUGN-001-04US-SeqList-ST25.txt NUGN_001_04US_SeqList_ST25.txt, date recorded: July 21, 2021 ~~November 12, 2014~~, file size 3 kilobytes).

REMARKS

I. Status as Continuation

The present application is being filed as a continuation of U.S. Application No. 16/743,604, which is currently pending. Therefore, the present application is co-pending with a prior-filed nonprovisional patent application in fulfillment of the requirements of 35 U.S.C. §120.

II. Amendments to the Specification

By this preliminary amendment, Applicant has amended the specification to recite the continuing data for the present application and to update the sequence listing name. Applicant submits that these amendments to the specification introduce no new matter.

III. Sequence Listing

Applicant is filing herewith a Sequence Listing in computer readable form in the ASCII text file named: NUGN-001-04US-SeqList-ST25.txt. The Sequence Listing is 3 kilobytes in size, was created on July 21, 2021 in the IBM-PC machine format, and having an operating system compatibility with MS-Windows.

The sequence listing has been updated relative to that filed in U.S. 16/743,604 to include all priority application information for this continuation. Applicant is also filing herewith a Statement of Compliance pursuant to 37 C.F.R. §1.825 indicating that the Sequence Listing contains no new matter. *See, e.g., MPEP (9th ed. Rev. 08.2017), §608.05.*

In accordance with MPEP §2422.03(a), the ASCII text file of the Sequence Listing will serve as both the paper copy and the computer readable form (CRF) of the Sequence Listing. Accordingly, Applicant is not submitting herewith: (1) a second copy of the sequence listing as a PDF file; (2) a statement under 37 C.F.R. §1.821(f) indicating that the paper copy and CRF of the sequence listing are identical; or (3) a request to use a compliant computer readable form of

the sequence listing that is already on file for another application pursuant to 37 C.F.R.

§1.821(e).

If there are any questions regarding these remarks, the Examiners are invited and encouraged to contact Applicant's representatives at the telephone number provided.

Dated: July 21, 2021

Respectfully submitted,

BROWN RUDNICK LLP

BROWN RUDNICK LLP

One Financial Center

Boston, MA 02111

Tel: 617-856-8483

/Thomas C. Meyers/

Thomas C. Meyers, Reg. No. 36,989

Attorney for Applicant

Email: tmeyers@brownrudnick.com

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Tecan Genomics, Inc.	ART UNIT:	Not Yet Assigned
SERIAL NUMBER:	Continuation of US Serial No. 16/743,604	CONF. NO.:	Not Yet Assigned
FILING DATE:	Herewith	EXAMINER:	Not Yet Assigned
TITLE:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

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STATEMENT OF COMPLIANCE UNDER 37 C.F.R. §1.825

In accordance with 37 C.F.R. §1.825, I hereby certify that the content of the Sequence Listing submitted herewith as an ASCII text file named NUGN-001-04US-SeqList-ST25.txt does not introduce any new matter into the subject application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued therefrom.

Dated: July 21, 2021

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: 212-209-4988

Respectfully submitted,
BROWN RUDNICK LLP

/Robert B. Ruh III/
Robert B. Ruh III, Reg. No. 73,996

Electronic Patent Application Fee Transmittal

Application Number:				
Filing Date:				
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ			
First Named Inventor/Applicant Name:	Douglas A. Amorese			
Filer:	Thomas C. Meyers/Sandra Richards			
Attorney Docket Number:	NUGN-001/04US 34079/550			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
UTILITY APPLICATION FILING	1011	1	320	320
UTILITY SEARCH FEE	1111	1	700	700
UTILITY EXAMINATION FEE	1311	1	800	800
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	1202	1	100	100
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				1920

Electronic Acknowledgement Receipt

EFS ID:	43309891
Application Number:	17381888
International Application Number:	
Confirmation Number:	6188
Title of Invention:	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ
First Named Inventor/Applicant Name:	Douglas A. Amorese
Customer Number:	21710
Filer:	Thomas C. Meyers/Sandra Richards
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	NUGN-001/04US 34079/550
Receipt Date:	21-JUL-2021
Filing Date:	
Time Stamp:	16:13:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$ 1920
RAM confirmation Number	E20217KG14421422
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

QIAGEN Exhibit 1012.0224

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Sequence Listing (Text File)	NUGN-001-04US-SeqList_ST25.txt	2694	no	-
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Information:					
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	Specification		1	40	
	Claims		41	43	
	Abstract		44	44	
Warnings:					
Information:					
4	Drawings-only black and white line drawings	NUGN-001-04US--Drawings.pdf	4460762	no	6
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Warnings:					
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5	Oath or Declaration filed	NUGN-001-01US-Declarations.pdf	339248	no	3
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QIAGEN Exhibit 1012.0225					

Warnings:					
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Total Files Size (in bytes):			8243015		

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National Stage of an International Application under 35 U.S.C. 371

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New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	NUGN-001/04US 34079/550
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
--------------------------	---

Inventor Information:

Inventor	1				Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Douglas	A.	Amorese		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Los Altos	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	900 Chesapeake Drive				
Address 2					
City	Redwood City	State/Province	CA		
Postal Code	94063	Country	US		

Inventor	2				Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Jonathan		Scolnick		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	San Francisco	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	451 Kansas Street #518				
Address 2					
City	San Francisco	State/Province	CA		
Postal Code	94107	Country	US		

Inventor	3				Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Ben		Schroeder		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	NUGN-001/04US 34079/550
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		

City	San Mateo	State/Province	CA	Country of Residence	US
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Mailing Address of Inventor:

Address 1	33 Hayward Ave, Apt 112				
Address 2					
City	San Mateo	State/Province	CA		
Postal Code	94401	Country	US		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).			
☐ An Address is being provided for the correspondence information of this application.			
Customer Number	21710		
Email Address	ip@brownrudnick.com	Add Email	Remove Email

Application Information:

Title of the Invention	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		
Attorney Docket Number	NUGN-001/04US 34079/550	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	6	Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐ Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

QIAGEN Exhibit 1012.0229

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	NUGN-001/04US 34079/550
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	21710		

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This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	16/743604	2020-01-15
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
16/743604	Continuation of	15/406002	2017-01-13
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
15/406002	Division of	14/540917	2014-11-13
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14/540917	Claims benefit of provisional	61/903826	2013-11-13
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	NUGN-001/04US 34079/550
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	NUGN-001/04US 34079/550
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

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The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

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☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	NUGN-001/04US 34079/550
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant	1	Remove
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section. Clear		
☒ Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.		Person who shows sufficient proprietary interest
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:		
Name of the Deceased or Legally Incapacitated Inventor:		
If the Applicant is an Organization check here. ☒		
Organization Name	Tecan Genomics, Inc.	
Mailing Address Information For Applicant:		
Address 1	900 Chesapeake Drive	
Address 2		
City	Redwood City	State/Province CA
Country	US	Postal Code 94063
Phone Number		Fax Number
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	NUGN-001/04US 34079/550
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		

Assignee	1			
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.				
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See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Thomas C. Meyers/		Date (YYYY-MM-DD)	2021-07-21
First Name	Thomas	Last Name	Meyers	Registration Number
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	NUGN-001/04US 34079/550
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ		

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet 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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**COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE
SEQUENCING READ**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 61/903,826, filed November 13, 2013, which is herein incorporated by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: NUGN_001_01US_SeqList_ST25.txt, date recorded: November 12, 2014, file size 3 kilobytes).

FIELD OF THE PRESENT INVENTION

[0003] The present invention relates generally to the field of high throughput sequencing reactions and the ability to discern artifacts arising through sequence duplications from nucleotide molecules that are unique molecules.

BACKGROUND OF THE PRESENT INVENTION

[0004] In RNA sequencing applications, accurate gene expression measurements may be hampered by PCR duplicate artifacts that occur during library amplification. When analyzing RNA sequencing data, when two or more identical sequences are found, it can be difficult to know if these represent unique cDNA molecules derived independently from different RNA molecules, or if they are PCR duplicates derived from a single RNA molecule. In genotyping by

sequencing, duplicate reads can be considered non-informative and may be collapsed down to a single read, thus reducing the number of sequencing reads used in final analysis. Generally, sequencing reads may be determined to be duplicates if both forward and reverse reads have identical starting positions, even though two independently generated molecules can have identical starting positions by random chance. Single primer extension based targeted re-sequencing suffers from an issue in that only one end of a sequencing read is randomly generated, while the other (reverse read) end is generated by a specific probe. This may make it difficult to determine if two reads are duplicates because they have been duplicated by PCR or because by chance they happened to start at the same position.

[0005] In expression analysis studies there may be limited value in doing paired end sequencing since the goal of the experiment is to determine amounts of transcript present as opposed to studying exon usage. In these studies, paired end sequencing adds costs while the only value is in helping distinguish PCR duplicates. The probability of two reads starting in the same position on only one end is higher than the probability of two reads having the same starting position on two ends (forward and reverse read). There is a need for improved methods that allow for low-cost, high throughput sequencing of regions of interest, genotyping or simple detection of RNA transcripts without inherent instrument inefficiencies that drive up sequencing costs due to the generation of unusable or non-desired data reads. The invention described herein fulfills this need. Here, we describe an adaptor approach that allows for the identification of true PCR duplicates and their removal.

[0006] The methods of the present invention provide novel methods for identifying true duplicate reads during sequencing, such as to improve data analysis of sequencing data, and other related advantages.

SUMMARY OF THE PRESENT INVENTION

[0007] The present invention is based, in part, on compositions and methods for discerning duplicate sequencing reads from a population of sequencing reads. The detection and/or removal of duplicate sequencing reads presented herein is a novel approach to increasing the efficacy of evaluating data generated from high throughput sequence reactions, including complex multiplex sequence reactions.

[0008] Accordingly, the present invention provides a method of detecting a duplicate sequencing read from a population of sample sequencing reads, the method comprising ligating an adaptor to a 5' end of each nucleic acid fragment of a plurality of nucleic acid fragments from one or more samples, wherein the adaptor comprises an indexing primer binding site, an indexing site, an identifier site, and a target sequence primer binding site. The ligated adaptor-nucleic acid fragment products can be amplified, thus generating a population of sequencing reads from the amplified adaptor-nucleic acid ligation products. The sequencing reads with a duplicate identifier site and target sequence can then be detected from the population of sequencing reads. The methods can further include the removal of the sequencing reads with the duplicate identifier site and target sequence from the population of sequence reads.

[0009] In some embodiments, the identifier site is sequenced with the indexing site or the target sequence. In further embodiments, the identifier site is sequenced separately from the indexing site or the target sequence.

[0010] In some embodiments, the adaptor comprises from 5' to 3' the indexing primer binding site; the indexing site; the identifier site; and the target sequence primer binding site. In further embodiments, the adaptor comprises from 5' to 3' the indexing primer binding site; the indexing site; the target sequence primer binding site; and the identifier site.

[0011] In some embodiments, the plurality of nucleic acid fragments is generated from more than one sample. In some embodiments, the nucleic acid fragments from each sample have the same indexing site. In some embodiments, the sequencing reads are separated based on the indexing site. In yet other embodiments, the separation of sequencing reads is performed prior to detecting sequence reads with a duplicate identifier site and target sequence.

[0012] In some embodiments, the nucleic acid fragments are DNA fragments, RNA fragments, or DNA/RNA fragments. In further embodiments, the nucleic acid fragments are genomic DNA fragments or cDNA fragments.

[0013] In some embodiments, the indexing site is between 2 and 8 nucleotides in length. In further embodiments, the indexing site is about 6 nucleotides in length. In some embodiments, the identifier site is between 1 and 8 nucleotides in length. In further embodiments, the identifier site is about 8 nucleotides in length.

[0014] In some embodiments, the indexing primer binding site is a universal indexing primer binding site; and in some embodiments, the target sequence primer binding site is a universal target sequence primer binding site.

[0015] The present invention also encompasses embodiments that include a kit comprising a plurality of adaptors, wherein each adaptor comprise an indexing primer binding site; an indexing site, and identifier site, and a target sequencing primer binding site.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] A better understanding of the novel features of the invention and advantages of the present invention will be obtained by reference to the following description that sets forth

illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0017] **Figure 1** depicts a schematic of generating sequencing reads of a library including where an indexing primer and a target sequence primer anneal.

[0018] **Figure 2A** depicts a mechanism of the Single Primer Enrichment Technology (SPET) and how an identifier site is carried over into the final library and how sequencing through the indexing site into the identifier site provides data on which nucleic acid molecule is being identified.

[0019] **Figure 2B** is a continuation of Figure 2A.

[0020] **Figure 3** offers a detailed view of the sequence of an example adaptor, among many envisioned embodiments, and the position of the indexing and identifier sites (SEQ ID NO: 1). “N” refers to any nucleic acid.

[0021] **Figure 4** depicts a schematic of two separate sequence libraries, indicating where the indexing primers and the target primers anneal in a traditional library as compared to a library using an identifier site.

[0022] **Figure 5** depicts a data table demonstrating the accuracy of resolving true duplicates versus apparent or perceived duplicates using an identifier site in the adaptors.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0023] The present invention is based, in part, on compositions and methods for discerning duplicate sequencing reads from a population of sequencing reads. The present invention encompasses methods of detecting sequences duplicated in sequencing applications, and further removal of the duplicated sequence reads. The present invention further encompasses kits

comprising components that would allow for customized applications of the method of detecting and removing duplicated sequence reads in high throughput sequencing reactions. The compositions and methods can be used with various applications for genetic sample analysis, such as RNA sequence analysis, copy number variation analysis, methylation sequencing analysis, genotyping and whole genome amplification.

[0024] Reference will now be made in detail to exemplary embodiments of the invention. While the disclosed methods and compositions will be described in conjunction with the exemplary embodiments, it will be understood that these exemplary embodiments are not intended to limit the present invention. On the contrary, the present disclosure is intended to encompass alternatives, modifications and equivalents, which may be included in the spirit and scope of the present invention.

[0025] Unless otherwise specified, terms and symbols of genetics, molecular biology, biochemistry and nucleic acid used herein follow those of standard treatises and texts in the field, e.g. Kornberg and Baker, *DNA Replication*, Second Edition (W.H. Freeman, New York, 1992); Lehninger, *Biochemistry*, Second Edition (Worth Publishers, New York, 1975); Strachan and Read, *Human Molecular Genetics*, Second Edition (Wiley-Liss, New York, 1999); Eckstein, editor, *Oligonucleotides and Analogs: A Practical Approach* (Oxford University Press, New York, 1991); Gait, editor, *Oligonucleotide Synthesis: A Practical Approach* (IRL Press, Oxford, 1984); and the like.

[0026] In some embodiments, the methods disclosed herein is for detecting from a population of sequencing reads a sequencing read, such as a duplicate sequencing read with a duplicate identifier site and target sequence. A duplicate sequencing read can be a sequencing read with

the same identifier site and target sequence as another sequencing read in the population of sequencing reads.

Adaptor

[0027] The present invention provides compositions of adaptors and methods comprising use of an adaptor. An adaptor refers to an oligonucleotide sequence, the ligation of which to a target polynucleotide or a target polynucleotide strand of interest enables the generation of amplification-ready products of the target polynucleotide or the target polynucleotide strand of interest. The target polynucleotide molecules may be fragmented or not prior to the addition of adaptors. In some embodiments, a method disclosed herein comprises ligating an adaptor to a 5' end of each nucleic acid fragment of a plurality of nucleic acid fragments from one or more samples.

[0028] Various adaptor designs are envisioned which are suitable for generation of amplification-ready products of target sequence regions/strands of interest. For example, the two strands of the adaptor may be self-complementary, non-complementary or partially complementary. In some embodiments, the adaptor can comprise an indexing primer binding site, an indexing site, an identifier site, and a target sequence primer binding site.

[0029] An indexing primer binding site is a nucleotide sequence for binding a primer for an indexing site. An indexing site is a nucleic acid sequence that acts as an index for multiple polynucleotide samples, thus allowing for the samples to be pooled together into a single sequencing run, which is known as multiplexing. In some embodiments, the indexing site is at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In some embodiments, the indexing site is between 2 and 8 nucleotides in length. In some embodiments, the indexing site is about 6 nucleotides in length.

[0030] An identifier site is a nucleic acid sequence that comprises random bases and is used to identify duplicate sequencing reads. In some embodiments, the identifier site is at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In some embodiments, the identifier site is between 1 and 8 nucleotides in length. In some embodiments, the identifier site is about 8 nucleotides in length. This identifier site can be designed as a set of sequences, or it can be semi-random, or it can be completely random. In addition, this identifier site can be a fixed length, or it can be a variable length. In some embodiments, the identifier sites in a plurality of adaptors are of a fixed length. For example, the identifier sites can all be eight random bases. In another embodiment, the identifier sites in a plurality of adaptors are of a variable length. For example, the identifier sites can range in size from 1 to 8 bases. In yet another embodiment, the identifier sites can be of a defined set of defined sequence. For example, the identifier site of a plurality of adaptors can be one of 96 defined six-base nucleotide sequences.

[0031] A target sequence primer binding site nucleotide sequence for binding a primer for a target sequence. The primer can be used to amplify the target sequence (e.g., a nucleic acid fragment from a sample). Accordingly, in some embodiments, the adaptor comprises an indexing primer binding site and a target sequence primer binding site.

[0032] A primer is a polynucleotide chain, typically less than 200 residues long, most typically between 15 and 100 nucleotides long, but can encompass longer polynucleotide chains. A primer targeting the primer binding sites are typically designed to hybridize to single-stranded nucleic acid strands. In some embodiments, the primers targeting the primer binding sites are designed to hybridize to single-stranded DNA targets. In the case where the sample comprises genomic DNA or other double-stranded DNA, the sample can be first denatured to render the target single stranded and enable hybridization of the primers to the desired sequence regions of

interest. In these embodiments, the methods and compositions described herein can allow for region-specific enrichment and amplification of sequence regions of interest. In some embodiments, the other double-stranded DNA can be double-stranded cDNA generated by first and second strand synthesis of one or more target RNAs.

[0033] In other embodiments, the primers targeting the primer binding sites are designed to hybridize to double-stranded nucleic acid targets, without denaturation of the double stranded nucleic acids. In other embodiments, the primers targeting the primer binding sites are designed to hybridize to a double-stranded DNA target, without denaturation of the dsDNA. In these embodiments, the primers targeting the selected sequence regions of interest are designed to form a triple helix (triplex) at the selected sequence regions of interest. The hybridization of the primers to the double-stranded DNA sequence regions of interest can be carried out without prior denaturation of the double stranded nucleic acid sample. In such embodiments, the methods and compositions described herein can allow for region-specific enrichment as well as strand-specific enrichment and amplification of sequence regions of interest. This method can be useful for generation of copies of strand specific sequence regions of interest from complex nucleic acid without the need to denature the dsDNA input DNA, thus enabling enrichment and analysis of multiplicity of sequence regions of interest in the native complex nucleic acid sample. The method can find use for studies and analyses carried out in situ, enable studies and analysis of complex genomic DNA in single cells or collection of very small well defined cell population, as well as permit the analysis of complex genomic DNA without disruption of chromatin structures.

[0034] The primers of the invention are generally oligonucleotides that are employed in an extension reaction by a polymerase along a polynucleotide template, such as for amplification of a target sequence (e.g., in PCR). The oligonucleotide primer can be a synthetic polynucleotide

that is single stranded, containing a sequence at its 3'-end that is capable of hybridizing with a sequence of the target polynucleotide. In some embodiments, the 3' region of the primer that hybridizes with the target nucleic acid has at least 80%, preferably 90%, more preferably 95%, most preferably 100%, complementarity to a primer binding site.

[0035] In some embodiments, the primer binding site is a binding site for a universal primer. A universal primer is a primer that can be used for amplifying a number of different sequences. In some embodiments, a universal primer is used to amplify different libraries. In some embodiments, an indexing primer binding site is a binding site for a universal indexing primer (i.e., the indexing primer binding site is a universal indexing primer binding site). In some embodiments, the adaptors used for ligating a plurality of nucleic acid fragments have a universal indexing primer binding site. In some embodiments, the universal indexing primer can be used to amplify and/or sequence a number of different indexing sites.

[0036] In some embodiments, a target sequence primer binding site is a binding site for a universal target sequence primer (i.e., the target sequence primer binding site is a universal target sequence primer binding site). In some embodiments, the adaptors used for ligating a plurality of nucleic acid fragments have a universal target sequence primer binding site. In some embodiments, the universal target sequence primer can be used to amplify and/or sequence a number of different target sequences.

[0037] In some embodiments, the adaptor comprises an identifier site 3' to an indexing site. In some embodiments, the adaptor comprises an identifier site 5' to an indexing site. In some embodiments, the adaptor comprises from 5' to 3' an indexing primer binding site, indexing site, identifier site, and target sequence primer binding site. In other embodiments, the adaptor comprises from 5' to 3' an indexing primer binding site, indexing site, identifier site, and target

sequence primer binding site. In yet other embodiments, the adaptor comprises from 5' to 3' an indexing primer binding site, identifier site, indexing site, and target sequence primer binding site.

Samples

[0038] In some embodiments, an adaptor is ligated to a nucleic acid fragment (e.g., the 5' end of the nucleic acid fragment). The nucleic acid fragment can be from a plurality of nucleic acid fragments from one or more samples. The nucleic acid fragment can be RNA, DNA, or complex DNA, for example genomic DNA and PNA, in which case one might use a modified nucleic acid. The nucleic acid fragment may also be cDNA. The cDNA can be generated from RNA, e.g., mRNA.

[0039] The sample can be a biological sample. For example, the sample can be an animal, plant, bacterial, algal, or viral sample. In some embodiments, the sample is a human, rat, or mouse sample. The sample can be from a mixture of genomes of different species such as host-pathogen, bacterial populations and the like. The sample can be cDNA made from a mixture of genomes of different species. In some embodiments, the sample can be from a synthetic source. The sample can be mitochondrial DNA. The sample can be cell-free DNA. The cell-free DNA can be obtained from sources such as a serum or a plasma sample. The sample can comprise one or more chromosomes. For example, if the sample is from a human, the sample can comprise one or more of chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X, or Y. In some embodiments, the sample comprises a linear or circular genome. The sample can be plasmid DNA, cosmid DNA, bacterial artificial chromosome (BAC), or yeast artificial chromosome (YAC). The sample can be from more than one individual or organism. The sample

can be double-stranded or single-stranded. The sample can be part of chromatin. The sample can be associated with histones.

[0040] In some embodiments, adaptors are ligated to a plurality of nucleic acid fragments from more than one sample, such as 2, 3, 4, 5, or more samples. In some embodiments, the nucleic acid fragments from each sample have the same indexing site. In some embodiments, a plurality of nucleic acid fragments is generated from a first sample and a second sample and adaptors are ligated to each nucleic acid fragment, in which adaptors ligated to each nucleic acid fragment from the first sample have the same first indexing site and adaptors ligated to nucleic acid fragments from the second sample has the same second indexing site. In some embodiments, the nucleic acid fragments or data associated with the nucleic acid fragments (e.g., sequencing reads) are separated based on the indexing site.

[0041] In some embodiments, a population of nucleic acid fragments generated from a sample is of one or more specific size range(s). In some embodiments, the fragments have an average length from about 10 to about 10,000 nucleotides. In some embodiments, the fragments have an average length from about 50 to about 2,000 nucleotides. In some embodiments, the fragments have an average length from about 100-2,500, 10-1,000, 10-800, 10-500, 50-500, 50-250, or 50-150 nucleotides. In some embodiments, the fragments have an average length less than 10,000 nucleotide, such as less than 5,000 nucleotides, less than 2,500 nucleotides, less than 2,500 nucleotides, less than 1,000 nucleotides, less than 500 nucleotides, such as less than 400 nucleotides, less than 300 nucleotides, less than 200 nucleotides, or less than 150 nucleotides.

[0042] In some embodiments, fragmentation of the nucleic acids can be achieved through methods known in the art. Fragmentation can be achieved through physical fragmentation methods and/or enzymatic fragmentation methods. Physical fragmentation methods can include

nebulization, sonication, and/or hydrodynamic shearing. In some embodiments, the fragmentation can be accomplished mechanically comprising subjecting the nucleic acids in the input sample to acoustic sonication. In some embodiments, the fragmentation comprises treating the nucleic acids in the input sample with one or more enzymes under conditions suitable for the one or more enzymes to generate double-stranded nucleic acid breaks. Examples of enzymes useful in the generation of nucleic acid or polynucleotide fragments include sequence specific and non-sequence specific nucleases. Non-limiting examples of nucleases include DNase I, Fragmentase, restriction endonucleases, variants thereof, and combinations thereof. Reagents for carrying out enzymatic fragmentation reactions are commercially available (e.g., from New England Biolabs). For example, digestion with DNase I can induce random double-stranded breaks in DNA in the absence of Mg^{++} and in the presence of Mn^{++} . In some embodiments, fragmentation comprises treating the nucleic acids in the input sample with one or more restriction endonucleases. Fragmentation can produce fragments having 5' overhangs, 3' overhangs, blunt ends, or a combination thereof. In some embodiments, such as when fragmentation comprises the use of one or more restriction endonucleases, cleavage of sample polynucleotides leaves overhangs having a predictable sequence. In some embodiments, the method includes the step of size selecting the fragments via standard methods known in the art such as column purification or isolation from an agarose gel.

[0043] In some embodiments, fragmentation of the nucleic acids is followed by end repair of the nucleic acid fragments. End repair can include the generation of blunt ends, non-blunt ends (i.e. sticky or cohesive ends), or single base overhangs such as the addition of a single dA nucleotide to the 3'-end of the nucleic acid fragments, by a polymerase lacking 3'-exonuclease activity. End repair can be performed using any number of enzymes and/or methods known in the art

including, but not limited to, commercially available kits such as the Encore™ Ultra Low Input NGS Library System I. In some embodiments, end repair can be performed on double stranded DNA fragments to produce blunt ends wherein the double stranded DNA fragments contain 5' phosphates and 3' hydroxyls. In some embodiments, the double-stranded DNA fragments can be blunt-end polished (or "end-repaired") to produce DNA fragments having blunt ends, prior to being joined to adapters. Generation of the blunt ends on the double stranded fragments can be generated by the use of a single strand specific DNA exonuclease such as for example exonuclease 1, exonuclease 7 or a combination thereof to degrade overhanging single stranded ends of the double stranded products. Alternatively, the double stranded DNA fragments can be blunt ended by the use of a single stranded specific DNA endonuclease, for example, but not limited to, mung bean endonuclease or S1 endonuclease. Alternatively, the double stranded products can be blunt ended by the use of a polymerase that comprises single stranded exonuclease activity such as for example T4 DNA polymerase, or any other polymerase comprising single stranded exonuclease activity or a combination thereof to degrade the overhanging single stranded ends of the double stranded products. In some cases, the polymerase comprising single stranded exonuclease activity can be incubated in a reaction mixture that does or does not comprise one or more dNTPs. In other cases, a combination of single stranded nucleic acid specific exonucleases and one or more polymerases can be used to blunt end the double stranded fragments generated by fragmenting the sample comprising nucleic acids. In still other cases, the nucleic acid fragments can be made blunt ended by filling in the overhanging single stranded ends of the double stranded fragments. For example, the fragments may be incubated with a polymerase such as T4 DNA polymerase or Klenow polymerase or a combination thereof in the presence of one or more dNTPs to fill in the single stranded portions

of the double stranded fragments. Alternatively, the double stranded DNA fragments can be made blunt by a combination of a single stranded overhang degradation reaction using exonucleases and/or polymerases, and a fill-in reaction using one or more polymerases in the presence of one or more dNTPs.

[0044] U.S. Patent Publication Nos. 2013-0231253 A1 and 2014-0274729 A1 further describe methods of generating nucleic acid fragments, methods of modifying the fragments and analysis of the fragments, and are incorporated by reference in their entirety herein.

Ligation of Adaptors

[0045] Ligation of adaptors at the desired end of the sequence regions of interest (e.g., at the 5' or 3' end of a nucleic acid fragment generated from a sample) is suitable for carrying out the methods of the invention. Various ligation modalities are envisioned, dependent on the choice of nucleic acid, nucleic acid modifying enzymes and the resulting ligatable end of the nucleic acid. For example, when a blunt end product comprising the target region/sequence of interest is generated, blunt end ligation can be suitable. Alternatively, where the cleavage is carried out using a restriction enzyme of known sequence specificity, leading to the generation of cleavage sites with known sequence overhangs, suitable ends of the adaptors can be designed to enable hybridization of the adaptor to the cleavage site of the sequence region of interest and subsequent ligation. Ligation also refers to any joining of two nucleic acid molecules that results in a single nucleic acid sequence that can be further modified to obtain the sequence of the nucleic acids in question. Reagents and methods for efficient and rapid ligation of adaptors are commercially available, and are known in the art.

[0046] In some embodiments, the 5' and/or 3' end nucleotide sequences of fragmented nucleic acids are not modified or end-repaired prior to ligation with the adapter oligonucleotides of the present invention. For example, fragmentation by a restriction endonuclease can be used to leave

a predictable overhang, followed by ligation with one or more adapter oligonucleotides comprising an overhang complementary to the predictable overhang on a nucleic acid fragment. In another example, cleavage by an enzyme that leaves a predictable blunt end can be followed by ligation of blunt-ended nucleic acid fragments to adapter oligonucleotides comprising a blunt end. In some embodiments, end repair can be followed by an addition of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleotides, such as one or more adenine, one or more thymine, one or more guanine, or one or more cytosine, to produce an overhang. Nucleic acid fragments having an overhang can be joined to one or more adapter oligonucleotides having a complementary overhang, such as in a ligation reaction. For example, a single adenine can be added to the 3' ends of end-repaired DNA fragments using a template independent polymerase, followed by ligation to one or more adapters each having a thymine at a 3' end. In some embodiments, adapter oligonucleotides can be joined to blunt end double-stranded nucleic acid fragments which have been modified by extension of the 3' end with one or more nucleotides followed by 5' phosphorylation. In some cases, extension of the 3' end can be performed with a polymerase such as for example Klenow polymerase or any of the suitable polymerases provided herein, or by use of a terminal deoxynucleotide transferase, in the presence of one or more dNTPs in a suitable buffer containing magnesium. In some embodiments, nucleic acid fragments having blunt ends can be joined to one or more adapters comprising a blunt end. Phosphorylation of 5' ends of nucleic acid fragments can be performed for example with T4 polynucleotide kinase in a suitable buffer containing ATP and magnesium. The fragmented nucleic acid molecules may optionally be treated to dephosphorylate 5' ends or 3' ends, for example, by using enzymes known in the art, such as phosphatases.

[0047] In some embodiments, appending the adaptor to the nucleic acid fragments generated by methods described herein can be achieved using a ligation reaction or a priming reaction. In some embodiments, appendage of an adaptor to the nucleic acid fragments comprises ligation. In some embodiments, ligation of the adaptor to the nucleic acid fragments can be following end repair of the nucleic acid fragments. In another embodiment, the ligation of the adaptor to the nucleic acid fragments can be following generation of the nucleic acid fragments without end repair of the nucleic acid fragments. The adaptor can be any type of adaptor known in the art including, but not limited to, conventional duplex or double stranded adaptors in which the adaptor comprises two complementary strands. In some embodiments, the adaptor can be a double stranded DNA adaptor. In some embodiments, the adaptor can be an oligonucleotide of known sequence and, thus, allow generation and/or use of sequence specific primers for amplification and/or sequencing of any polynucleotides to which the adaptor is appended or attached. In some embodiments, the adaptor can be a conventional duplex adaptor, wherein the adaptor comprises sequence well known in the art. In some embodiments, the adaptor can be appended to the nucleic acid fragments generated by the methods described herein in multiple orientations. In some embodiment, the methods described herein can involve the use of a duplex adaptor comprising double stranded DNA of known sequence that is blunt ended and can bind to the double stranded nucleic acid fragments generated by the methods described herein in one of two orientations. In some embodiments, the adaptor can be ligated to each of the nucleic acid fragments such that each of the nucleic acid fragments comprises the same adaptor. In other words, each of the nucleic acid fragments comprises a common adaptor. In another embodiment, an adaptor can be appended or ligated to a library of nucleic acid fragments generated by the methods described herein such that each nucleic acid fragment in the library of nucleic acid

fragments comprises the adaptor ligated to one or both ends. In another embodiment, more than one adaptor can be appended or ligated to a library of nucleic acid fragments generated by the methods described herein. The multiple adaptors may occur adjacent to one another, spaced intermittently, or at opposite ends of the nucleic acid fragments. In some embodiments, the adaptor can be ligated or appended to the 5' and/or 3' ends of the nucleic acid fragments generated by the methods described herein. The adaptor can comprise two strands wherein each strand comprises a free 3' hydroxyl group but neither strand comprises a free 5' phosphate. In some embodiments, the free 3' hydroxyl group on each strand of the adaptor can be ligated to a free 5' phosphate present on either end of the nucleic acid fragments of the present invention. In this embodiment, the adaptor comprises a ligation strand and a non-ligation strand whereby the ligation strand can be ligated to the 5' phosphate on either end of the nucleic acid fragment while a nick or gap can be present between the non-ligation strand of the adaptor and the 3' hydroxyl on either end of the nucleic acid fragment. In some embodiments, the nick or gap can be filled in by performing a gap repair reaction. In some embodiments, the gap repair can be performed with a DNA dependent DNA polymerase with strand displacement activity. In some embodiments, the gap repair can be performed using a DNA-dependent DNA polymerase with weak or no strand displacement activity. In some embodiments, the ligation strand of the adaptor can serve as the template for the gap repair or fill-in reaction. The gap repair or fill-in reaction may comprise an extension reaction wherein the ligation strand of the adaptor serves as a template and leads to the generation of nucleic acid fragments with complementary termini or ends. In some embodiments, the gap repair can be performed using Taq DNA polymerase. In some embodiments, the ligation of the first adaptor to the nucleic acid fragments generated by the

methods described herein may not be followed by gap repair. The nucleic acid fragments may comprise the adaptor sequence ligated only at the 5' end of each strand.

[0048] Ligation and, optionally gap repair, of the adaptor to the nucleic acid fragments generates an adaptor-nucleic acid fragment complex. In some embodiments, the adaptor-nucleic acid fragment complex can be denatured. Denaturation can be achieved using any of the methods known in the art including, but not limited to, physical, thermal, and/or chemical denaturation. In some embodiments, denaturation can be achieved using thermal or heat denaturation. In some embodiments, denaturation of the adaptor-nucleic acid fragment complex generates single stranded nucleic acid fragments comprising the adaptor sequence at only the 5' end of the nucleic acid fragments. In another embodiment, denaturation of the first adaptor-nucleic acid fragment complex generates single stranded nucleic acid fragments comprising adaptor sequence at both the 5' end and 3' end of the nucleic acid fragments.

Methods of Amplification

[0049] The methods, compositions and kits described herein can be useful to generate amplification-ready products directly from a nucleic acid source for downstream applications such as next generation sequencing, as well as generation of libraries with enriched population of sequence regions of interest. In some embodiments, the adapter-nucleic fragment ligated products, e.g., from the ligation of an adaptor to a 5' end of each nucleic acid fragment of a plurality of nucleic acid fragments from one or more samples, is amplified.

[0050] Methods of amplification are well known in the art. In some embodiments, the amplification is exponential, e.g. in the enzymatic amplification of specific double stranded sequences of DNA by a polymerase chain reaction (PCR). In other embodiments the amplification method is linear. In other embodiments the amplification method is isothermal. In

some embodiments, the amplification is exponential, e.g. in the enzymatic amplification of specific double stranded sequences of DNA by a polymerase chain reaction (PCR).

[0051] Suitable amplification reactions can be exponential or isothermal and can include any DNA amplification reaction, including but not limited to polymerase chain reaction (PCR), strand displacement amplification (SDA), linear amplification, multiple displacement amplification (MDA), rolling circle amplification (RCA), single primer isothermal amplification (SPIA, see e.g. U.S. Pat. No. 6,251,639), Ribo-SPIA, or a combination thereof. In some cases, the amplification methods for providing the template nucleic acid may be performed under limiting conditions such that only a few rounds of amplification (e.g., 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 etc.), such as for example as is commonly done for cDNA generation. The number of rounds of amplification can be about 1-30, 1-20, 1-15, 1-10, 5-30, 10-30, 15-30, 20-30, 10-30, 15-30, 20-30, or 25-30.

[0052] PCR is an in vitro amplification procedure based on repeated cycles of denaturation, oligonucleotide primer annealing, and primer extension by thermophilic template dependent polynucleotide polymerase, resulting in the exponential increase in copies of the desired sequence of the polynucleotide analyte flanked by the primers. The two different PCR primers, which anneal to opposite strands of the DNA, are positioned so that the polymerase catalyzed extension product of one primer can serve as a template strand for the other, leading to the accumulation of a discrete double stranded fragment whose length is defined by the distance between the 5' ends of the oligonucleotide primers.

[0053] LCR uses a ligase enzyme to join pairs of preformed nucleic acid probes. The probes hybridize with each complementary strand of the nucleic acid analyte, if present, and ligase is

employed to bind each pair of probes together resulting in two templates that can serve in the next cycle to reiterate the particular nucleic acid sequence.

[0054] SDA (Westin et al 2000, Nature Biotechnology, 18, 199-202; Walker et al 1992, Nucleic Acids Research, 20, 7, 1691-1696), is an isothermal amplification technique based upon the ability of a restriction endonuclease such as HincII or BsoBI to nick the unmodified strand of a hemiphosphorothioate form of its recognition site, and the ability of an exonuclease deficient DNA polymerase such as Klenow exo minus polymerase, or Bst polymerase, to extend the 3'-end at the nick and displace the downstream DNA strand. Exponential amplification results from coupling sense and antisense reactions in which strands displaced from a sense reaction serve as targets for an antisense reaction and vice versa.

[0055] Some aspects of the invention utilize linear amplification of nucleic acids or polynucleotides. Linear amplification generally refers to a method that involves the formation of one or more copies of the complement of only one strand of a nucleic acid or polynucleotide molecule, usually a nucleic acid or polynucleotide analyte. Thus, the primary difference between linear amplification and exponential amplification is that in the latter process, the product serves as substrate for the formation of more product, whereas in the former process the starting sequence is the substrate for the formation of product but the product of the reaction, i.e. the replication of the starting template, is not a substrate for generation of products. In linear amplification the amount of product formed increases as a linear function of time as opposed to exponential amplification where the amount of product formed is an exponential function of time.

Downstream Applications

[0056] One aspect of the present invention is that the methods and compositions disclosed herein can be efficiently and cost-effectively utilized for downstream analyses, such as in next generation sequencing or hybridization platforms, with minimal loss of biological material of interest. The methods of the present invention can also be used in the analysis of genetic information of selective genomic regions of interest (e.g., analysis of SNPs or other disease markers) as well as genomic regions which may interact with the selective region of interest. The methods of the present invention may further be used in the analysis of copy number variation as well as differential expression.

Sequencing

[0057] In some embodiments, a population of sequencing reads is generated from the amplified adapter-nucleic fragment ligated products. In some embodiments, a sequencing read comprises an index read, which comprises the sequence of an indexing site. In some embodiments, an index read comprises the sequence of an indexing site and the sequence of an identifier site. For example, the indexing site is sequenced with the identifier site. In some embodiments, the index read does not include the sequence of an identifier site. For example, the indexing site is not sequenced with the identifier site. In some embodiments, a sequencing read comprises the target sequence. In some embodiments, a sequencing read comprises a target sequence and an identifier sequence. For example, the target sequence is sequenced with the identifier site. In some embodiments, the target sequence is not sequenced with the identifier site.

[0058] The methods of the present invention are useful for sequencing by the method commercialized by Illumina, as described U.S. Pat Nos. 5,750,341; 6,306,597; and 5,969,119.

[0059] In general, double stranded fragment polynucleotides can be prepared by the methods of the present invention to produce amplified nucleic acid sequences tagged at one (e.g., (A)/(A')) or both ends (e.g., (A)/(A') and (C)/(C')). In some cases, single stranded nucleic acid tagged at one or both ends is amplified by the methods of the present invention (e.g., by SPIA or linear PCR). The resulting nucleic acid is then denatured and the single-stranded amplified polynucleotides are randomly attached to the inside surface of flow-cell channels. Unlabeled nucleotides are added to initiate solid-phase bridge amplification to produce dense clusters of double-stranded DNA. To initiate the first base sequencing cycle, four labeled reversible terminators, primers, and DNA polymerase are added. After laser excitation, fluorescence from each cluster on the flow cell is imaged. The identity of the first base for each cluster is then recorded. Cycles of sequencing are performed to determine the fragment sequence one base at a time.

[0060] In some embodiments, the methods of the invention are useful for preparing target polynucleotides for sequencing by the sequencing by ligation methods commercialized by Applied Biosystems (e.g., SOLiD sequencing). In other embodiments, the methods are useful for preparing target polynucleotides for sequencing by synthesis using the methods commercialized by 454/Roche Life Sciences, including but not limited to the methods and apparatus described in Margulies et al., *Nature* (2005) 437:376-380 (2005); and U.S. Pat. Nos. 7,244,559; 7,335,762; 7,211,390; 7,244,567; 7,264,929; and 7,323,305. In other embodiments, the methods are useful for preparing target polynucleotide(s) for sequencing by the methods commercialized by Helicos BioSciences Corporation (Cambridge, Mass.) as described in U.S. application Ser. No. 11/167,046, and U.S. Pat. Nos. 7,501,245; 7,491,498; 7,276,720; and in U.S. Patent Application Publication Nos. US20090061439; US20080087826; US20060286566; US20060024711;

US20060024678; US20080213770; and US20080103058. In other embodiments, the methods are useful for preparing target polynucleotide(s) for sequencing by the methods commercialized by Pacific Biosciences as described in U.S. Pat. Nos. 7,462,452; 7,476,504; 7,405,281; 7,170,050; 7,462,468; 7,476,503; 7,315,019; 7,302,146; 7,313,308; and US Application Publication Nos. US20090029385; US20090068655; US20090024331; and US20080206764.

[0061] An example of a sequencing technique that can be used in the methods of the provided invention is semiconductor sequencing provided by Ion Torrent (e.g., using the Ion Personal Genome Machine (PGM)). Ion Torrent technology can use a semiconductor chip with multiple layers, e.g., a layer with micro-machined wells, an ion-sensitive layer, and an ion sensor layer. Nucleic acids can be introduced into the wells, e.g., a clonal population of single nucleic can be attached to a single head, and the bead can be introduced into a well. To initiate sequencing of the nucleic acids on the beads, one type of deoxyribonucleotide (e.g., dATP, dCTP, dGTP, or dTTP) can be introduced into the wells. When one or more nucleotides are incorporated by DNA polymerase, protons (hydrogen ions) are released in the well, which can be detected by the ion sensor. The semiconductor chip can then be washed and the process can be repeated with a different deoxyribonucleotide. A plurality of nucleic acids can be sequenced in the wells of a semiconductor chip. The semiconductor chip can comprise chemical-sensitive field effect transistor (chemFET) arrays to sequence DNA (for example, as described in U.S. Patent Application Publication No. 20090026082). Incorporation of one or more triphosphates into a new nucleic acid strand at the 3' end of the sequencing primer can be detected by a change in current by a chemFET. An array can have multiple chemFET sensors.

[0062] Another example of a sequencing technique that can be used in the methods of the provided invention is nanopore sequencing (see e.g., Soni G V and Meller A. (2007) *Clin Chem*

53: 1996-2001). A nanopore can be a small hole of the order of 1 nanometer in diameter. Immersion of a nanopore in a conducting fluid and application of a potential across it can result in a slight electrical current due to conduction of ions through the nanopore. The amount of current that flows is sensitive to the size of the nanopore. As a DNA molecule passes through a nanopore, each nucleotide on the DNA molecule obstructs the nanopore to a different degree. Thus, the change in the current passing through the nanopore as the DNA molecule passes through the nanopore can represent a reading of the DNA sequence.

Data Analysis

[0063] In some embodiments, the sequence reads are used in the analysis of genetic information of selective genomic regions of interest as well as genomic regions which may interact with the selective region of interest. Amplification methods as disclosed herein can be used in the devices, kits, and methods known to the art for genetic analysis, such as, but not limited to those found in U.S. Pat. Nos. 6,449,562, 6,287,766, 7,361,468, 7,414,117, 6,225,109, and 6,110,709.

[0064] In some embodiments, the sequencing reads are used to detect duplicate sequencing reads. In some embodiments, a sequencing read is identified as a duplicate sequencing read when it contains an identifier site and target sequence that is the same as another sequencing read from the same population of sequencing reads.

[0065] In some embodiments, duplicate sequencing reads are differentiated from one another as being true duplicates versus apparent or perceived duplicates. Apparent or perceived duplicates may be identified from sequencing libraries and using conventional measures of duplicate reads (i.e., reads were mapped using bowtie), where all reads with the same start and end nucleic acid coordinates were counted as duplicates. True duplicates may be identified from sequencing

libraries having had an identifier site introduced through ligation to differentiate between fragments of DNA that randomly have the same start and end mapping coordinates.

[0066] In some embodiments, the sequence of the identifier sites from the index read of two nucleic acids generated from any dsDNA may have the same start site, as determined from the sequencing reads of the target sequence of the generated nucleic acid fragments. If the identifier site from the index read of the two nucleic acid fragments are not identical, then the target sequence reads were not generated from the same original dsDNA molecule and, therefore, are no true duplicate reads. The ligation of random sequences onto dsDNA molecules, accompanied by the methods of the invention, allow for the identification of true duplicate reads versus apparent or perceived duplicate reads.

[0067] In some embodiments, the sequence of the identifier sites from the index read of two nucleic acid fragments generated from a genomic DNA (gDNA) molecule that have the same start site, which can be determined from the sequencing reads of the target sequence of the nucleic acid fragments is determined. If the identifier site from the index read of the two nucleic acid fragments are not identical, then the target sequence reads were not generated from the same original gDNA molecule and, therefore, are not true duplicate reads. In another embodiment, an identifier site is inserted at the adapter insert junction. The sequence of the identifier site is carried through the library amplification step. The identifier site is the first sequence read during the forward read. As the identifier sequence is not logically present adjacent to naturally occurring sequence, this uniquely identifies the DNA fragment. Therefore, by ligating random sequences onto the original gDNA, the methods of the invention identify true duplicate reads.

[0068] In some embodiments, a duplicate sequencing read is detected and analyzed. Duplicate reads can be filtered using 'samtools rmdup', wherein reads with identical external coordinates

are removed, only one read with highest mapping quality is retained. After filtering, a filtered set of deduplicated reads can be used in any downstream analysis. Conversely, this filtering step can be skipped, and downstream analysis can be done using the unfiltered reads, including duplicates.

[0069] In some embodiments, sequencing reads are generated from a number of samples. In some embodiments, adaptors are ligated to a plurality of nucleic acid fragments from the samples, in which the nucleic acid fragments from each sample has the same indexing site. In some embodiments, a plurality of nucleic acid fragments is generated from a first sample and a second sample and adaptors are ligated to each nucleic acid fragment, in which adaptors ligated to each nucleic acid fragment from the first sample have the same first indexing site and adaptors ligated to nucleic acid fragments from the second sample has the same second indexing site. In some embodiments, the data associated with the nucleic acid fragments (e.g., sequencing reads) are separated based on the indexing site before sequencing reads of the target sequence and/or identifier site are analyzed. In some embodiments, the nucleic acid fragments or data associated with the nucleic acid fragments (e.g., sequencing reads) are separated based on the indexing site before duplicate sequencing reads are analyzed and/or removed.

[0070] In some embodiments, a method disclosed herein identifies or detects one or more true duplicate(s) with increased accuracy as compared to other methods. For example, in some embodiments, a method disclosed herein identify true duplicates (in contrast to identifying apparent or perceived duplicates) with increased accuracy as compared to other methods. The increased resolution and/or accuracy in identifying one or more true duplicate(s) can provide a considerable contribution to the state of the art in more accurately identifying true duplicates. In some embodiments, a method disclosed herein identifies or detects a true duplicate with

increased efficiency as compared to other methods, such as paired end sequencing. The increase in accuracy, resolution, and/or efficiency in detecting duplicate reads (e.g., true duplicate(s)) can increase confidence in sequencing results, such as for expression and CNV analysis.

Kits

[0071] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, the kit, in a suitable container, comprises: an adaptor or several adaptors, one or more of oligonucleotide primers and reagents for amplification.

[0072] The containers of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other containers, into which a component may be placed, and preferably, suitably aliquotted. Where there is more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a container.

[0073] When the components of the kit are provided in one or more liquid solutions, the liquid solution can be an aqueous solution. However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent.

[0074] A kit may include instructions for employing the kit components as well the use of any other reagent not included in the kit. Instructions may include variations that can be implemented.

[0075] In some embodiments, the invention provides kits containing any one or more of the elements disclosed in the above methods and compositions. In some embodiments, a kit comprises a composition of the invention, in one or more containers. In some embodiments, the

invention provides kits comprising adapters, primers, and/or other oligonucleotides described herein. In some embodiments, the kit further comprises one or more of: (a) a DNA ligase, (b) a DNA-dependent DNA polymerase, (c) an RNA-dependent DNA polymerase, (d) a forward adapter (e) one or more oligonucleotides comprising reverse adaptor sequence and (f) one or more buffers suitable for one or more of the elements contained in said kit. The adapters, primers, other oligonucleotides, and reagents can be, without limitation, any of those described above. Elements of the kit can further be provided, without limitation, in any of the amounts and/or combinations (such as in the same kit or same container) described above. The kits may further comprise additional agents, such as those described above, for use according to the methods of the invention. For example, the kit can comprise a first forward adaptor that is a partial duplex adaptor as described herein, a second forward adapter, and a nucleic acid modifying enzyme specific for a restriction and/or cleavage site present in the first forward adaptor. The kit elements can be provided in any suitable container, including but not limited to test tubes, vials, flasks, bottles, ampules, syringes, or the like. The agents can be provided in a form that may be directly used in the methods of the invention, or in a form that requires preparation prior to use, such as in the reconstitution of lyophilized agents. Agents may be provided in aliquots for single-use or as stocks from which multiple uses, such as in a number of reaction, may be obtained.

[0076] In some embodiments, the kit comprises a plurality of adaptor oligonucleotides, wherein each of the adaptor oligonucleotides comprises at least one of a plurality of identifier site sequences, wherein each identifier site sequence of the plurality of identifier site sequences differs from every other identifier site sequence in said plurality of identifier site sequences at at least three nucleotide positions, and instructions for using the same. Adapters comprising

different identifier site sequences can be supplied individually or in combination with one or more additional adapters having a different identifier site sequence. In some embodiments, the kit can comprises a plurality of adapter oligonucleotides.

EXAMPLES

Example 1: Identification of Duplicate Sequencing Reads with NuGEN Ovation Target Enrichment Library System

[0077] Sample Description: 100ng of DNA from a human HapMap sample (NA19238) was fragmented to approximately 500 base pair in length by sonication with a Covaris system (Covaris, Inc., Woburn, MA). The resulting DNA was treated with end repair enzyme mix NuGEN R01280 and R01439 (NuGEN Technologies, Inc., San Carlos, CA) according to supplier's recommendation to produce blunt ended DNA fragments.

[0078] Library Generation, Enrichment, and Identifier Site Incorporation: An oligonucleotide with segments, from 5' to 3' of the top strand, 1) an Illumina indexing read priming site such as AGAGCACACGTCTGAACTCCAGTCAC (SEQ ID NO:2), 2) an indexing site, 3) an identifier site having a random 6 base sequence and, 4) a sequence compatible with an Illumina forward sequencing priming site such as TCTTTCCCTACACGACGCTCTTCCGATCT (SEQ ID NO:3), was annealed to a second oligonucleotide to form a partially double stranded DNA adapter. Five uM of these adapters were ligated onto the end-repaired DNA using Ligase and Ligase reaction buffer from NuGEN's Ovation Ultralow Library System (NuGEN Technologies, Inc., San Carlos, CA) according to supplier's recommendations. Following 30 minutes of incubation at 25°C, the reaction mixture was diluted with water, 0.8X volume of Ampure XP magnetic beads (Agencourt Biosciences Corporation, A Beckman Coulter Company, Beverly, MA) was added and the solution thoroughly mixed. The beads were collected, washed and the ligated

DNA fragments eluted according to manufacturer's recommendations. A pool of targeting probes was annealed to the eluted DNA fragments by initially heating the solution to 95°C then slowly cooling the mixture from 80°C to 60°C by 0.6 degrees/minute. Targeting probes that were specifically annealed were extended with Taq DNA polymerase (New England Biolabs, Inc., Ipswich, MA) according to the manufacturer's protocols. Following extension, the DNA fragments were collected on Agencourt magnetic beads, washed and eluted according to manufacturer's recommendations. These libraries were enriched by 30 cycles of PCR using NuGEN library enrichment primers (Ovation Target Enrichment Library System, NuGEN Technologies, Inc., San Carlos, CA) that also contain the Illumina flow cell sequences (Illumina Inc., San Diego, CA) according to supplier's recommendation.

[0079] The resulting libraries were quantitated by qPCR using a kit provided by KAPA, diluted to 2nM and applied to an Illumina MiSeq DNA Sequencer (Illumina Inc., San Diego, CA). The following series was run: 36 base first read, 14 base second read, and 24 base third read.

[0080] Data Analysis: The sequencer output was processed in accordance with manufacturer's recommendation. In order to analyze the data, the indexing read was split into two files. The first file contained the first 8 bases of the indexing reads and is utilized as the library index file for standard library parsing. The other file contains only the random bases and is set aside for further sequence parsing.

[0081] Following our data analysis pipeline of sequence alignment with bowtie aligner (Langmead B. et al., Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* 2009, 10:R2.), duplicate reads were identified by their genomic start positions. At this point, sequencing reads that started at the same genomic position were checked against the random bases file to see if they have the same or different set of random

bases that were ligated to them. Where two sequences with the same starting genomic coordinate had the same set of random bases, they were considered to have come from the same initial DNA ligation event, regardless of which Ovation Target Enrichment targeting probe was used to generate the sequence fragment in question. These sequences, therefore, did not provide unique information about the starting genomic DNA and are considered as one sequencing read for the purpose of variant analysis. Two sequence reads with the same starting genomic coordinate that have different random bases were derived from unique ligation events and were considered to both be valid sequencing reads for the purpose of variant identification. **Figure 5** provides the analysis results demonstrating the identification of the duplicate reads. The use of an identifier site allowed for the determination of the number of true duplications versus the number of apparent or perceived duplicates.

[0082] If the sequences of two libraries are identical, their duplication status is unknown since this could occur by chance in any library. If an identifier sequence is used in combination with the library reads, the status can be determined (duplicates if identical, distinct if different). With the SPET system, one end is common so the probability of two libraries having identical ends increases. These would appear to be duplicate sequences and their true status can be determined by looking at an identifier sequence.

[0083] Over the sampling of all randomly selected reads, the use of identifier sites provided an increased resolution on the presence of true duplicates. When evaluating two million random reads, the apparent duplicates were found to comprise 39% of all reads. However, the true duplicates, identified through the use of an identifier site, were found to comprise only 26% of all reads. The methods employing the use of an identifier site were found to considerably increase the resolution of the true number of duplicates within the pool of reads.

Example 2: Removal of Duplicate Sequencing Reads with 8 Base Identifier Site

[0084] In a standard RNA sequencing library, adaptors are ligated to the ends of double stranded cDNA. These adaptors contain universal sequences that allow for PCR amplification and sequencing on high throughput sequencing machines. The adaptors are synthesized with a large population of additional sequences, in which each additional sequence is an identifier site, at the ligation end. The identifier sites are present at the junction between the adaptor and the cDNA. The sequence read starts with the identifier site and follows with the cDNA sequence.

[0085] This pool of identifier sites is used for the detection of PCR duplicates, as PCR duplicates will contain the identical identifier site, whereas two different cDNA molecules will ligate to two different adaptors containing two different identifier sites. This identifier site is designed as eight random bases introduced onto the end of the adaptor. Sequence reads from libraries made with such adaptors contain the 8 bases of the identifier site followed by the cDNA sequence. Standard PCR duplicate removal software such as PICARD, Markduplicates, and/or SAMtools rmdup is used to identify and remove the PCR duplicates, leaving behind for analysis any instances of multiple cDNA fragments that happen to have the same sequence.

Example 3: Removal of Duplicate Sequencing Reads with Mixture of Random 1-8 Base Identifier Sites

[0086] In a standard RNA sequencing library, adaptors are ligated to the ends of double stranded cDNA. These adaptors contain universal sequences that allow for PCR amplification and sequencing on high throughput sequencing machines. The adaptors are synthesized with a large population of additional sequences, in which each additional sequence is an identifier site, at the

ligation end. The identifier sites are present at the junction between the adaptor and the cDNA. The sequence read starts with the identifier site and follows with the cDNA sequence.

[0087] This pool of identifier sites is used for the detection of PCR duplicates, as PCR duplicates will contain the identical identifier site, whereas two different cDNA molecules will ligate to two different adaptors containing two different identifier sites.

[0088] One to eight random bases are introduced onto the end of the adaptor. Sequence reads from libraries made with such adaptors contain between 1 and 8 bases of the identifier site followed by the cDNA sequence. Standard PCR duplicate removal software such as PICARD, Markduplicates, and/or SAMtools rmdup is used to identify and remove the PCR duplicates, leaving behind for analysis any instances of multiple cDNA fragments that happen to have the same sequence.

Example 4: Removal of Duplicate Sequencing Reads with Mixture of 96 Defined 6-Base Identifier Sites

[0089] In a standard RNA sequencing library, adaptors are ligated to the ends of double stranded cDNA. These adaptors contain universal sequences that allow for PCR amplification and sequencing on high throughput sequencing machines. The adaptors are synthesized with a large population of additional sequences, in which each additional sequence is an identifier site, at the ligation end. The identifier sites are present at the junction between the adaptor and the cDNA. The sequence read starts with the identifier site and follows with the cDNA sequence.

[0090] This pool of identifier sites is used for the detection of PCR duplicates, as PCR duplicates will contain the identical identifier site, whereas two different cDNA molecules will ligate to two different adaptors containing two different identifier sites.

[0091] A mixture of 96 defined six-bases sequences are introduced onto the end of the adaptors. Thus, each six-base sequence is an identifier site. Sequence reads from libraries made with such adaptors contain one of the 96 six-base identifier site followed by the cDNA sequence. Standard PCR duplicate removal software such as PICARD, Markduplicates, and/or SAMtools rmdup is used to identify and remove the PCR duplicates, leaving behind for analysis any instances of multiple cDNA fragments that happen to have the same sequence.

Example 5: Identification of Duplicate Sequencing Reads in Determining mRNA Expression Levels

[0092] Sample Description: Total RNA is extracted from tumor and normal adjacent tissue for the purpose of finding differences in expression levels of transcripts between the two sample types. 100ng of each sample is converted into cDNA using the USP primers, reaction buffer, and Reverse Transcriptase provided in NuGEN's Encore Complete Library System (NuGEN Technologies, Inc., San Carlos, CA) according to the supplier's recommendations. This is followed by second strand synthesis, again using materials provided in the kit according to recommendations. Double stranded cDNA was prepared using the SuperScript® Double-Stranded cDNA Synthesis Kit from Life Technologies (Carlsbad, CA) according the manufacturer's instructions. DNA was sheared with a Covaris S-series device (Covaris, Inc., Woburn, MA) using the 200 bp sonication protocol provided with the instrument (10% duty cycle, 200 cycles/burst, 5 intensity, 180 seconds). DNA was treated with 1.5 uL 10X Blunting Buffer, 0.5 uL Blunting Enzyme (New England Biolabs, Inc., Ipswich, MA; p/n E1201) and 1.2 uL of 2.5mM of each dNTP mix in a total volume of 15 uL for 30 minutes at 25°C followed by 10 minutes at 70°C.

[0093] Library Generation: The DNA fragments were then subjected to end repair using end repair buffers and enzymes provided in NuGEN's Ovation Ultralow Library System (NuGEN Technologies, Inc., San Carlos, CA).

[0094] Forward adaptor 5'

[0095] AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCC
GATCTNNNNNNNN (SEQ ID NO:4),

[0096] Reverse adaptor 1) 5'

[0097] CAAGCAGAAGACGGCATACGAGATTCCCTTGTGACTGGAGTTCAGACGTGTG
CTCTTCCGATCTNNNNNNNN (SEQ ID NO:5),

[0098] Reverse adaptor 2) 5'

[0099] CAAGCAGAAGACGGCATACGAGATTGAAGGTGACTGGAGTTCAGACGTGT
GCTCTTCCGATCNNNNNNNN (SEQ ID NO:6), and

[00100] Common partner 5' NNNNNNNNAGATCGGAAGAGC (SEQ ID NO:7) were all ordered from IDT (Integrated DNA Technologies, Coralville, IA). The reverse adaptors each contain a unique identifier (underlined) that enables libraries made with these adapters to be distinguished. In this case N represents an equimolar mixture of A, C, G, and T. A mixture of 5uM forward, 5uM reverse, and 10uM common in 10mM MgCl₂, 50mM Tris pH 8 was heated to 95C for 5 minutes, then cooled to 20C. Adaptor ligation was performed by addition of 4.5 uL water, 3 uL Adaptor mix (prepared above), 6 uL 5X NEBNext Quick Ligation Reaction Buffer and 1.5 uL Quick T4 DNA Ligase (New England Biolabs, Inc., Ipswich, MA; p/n E6056), followed by incubation for 30 minutes at 25°C followed by 10 minutes at 70°C. Ligation products were purified by adding 70uL water and 80 uL of Ampure XP beads (Agencourt Genomics), washing twice with 70% ethanol and eluting with 20 uL of 10mM Tris pH 8.0.

Library products were amplified in a 50uL PCR containing 0.5uM each of primer (5' AATGATACGGCGACCACCGA (SEQ ID NO:8) , and 5' CAAGCAGAAGACGGCATACGA (SEQ ID NO:9), 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl₂, 0.2mM each dNTP, and 1 unit Taq polymerase. The reaction was cycled 15 times under the conditions 95C for 15 seconds, 60C for 1 minute. PCR products were purified with 1 volume of Ampure XP beads (Agencourt Biosciences Corporation, A Beckman Coulter Company, Beverly, MA) as described above. The library was analyzed by HS DNA Bioanalyzer (Agilent Technologies, Santa Clara, CA) and quantitated with the KAPA Library Quantification Kit (KAPA Biosystems, Wilmington, MA; p/n KK4835) according to the supplied instructions. The resulting libraries are combined and are compatible with standard TruSeq single end or paired Illumina sequencing protocols for GAIIx, MiSeq, or Hi Seq sequencing instruments (Illumina Inc., San Diego, CA). The following series is run; 50 base first read, 6 base second read. A third read is not required for counting purposes or duplication analysis.

[00101] Data Analysis: The sequencer output was processed in accordance with manufacturer's recommendation. The 6 bases of the index read is used for standard library parsing, separating the data files from the two sample types. The first 50 bases of the target sequence read are compared to each other. Any read that has identical sequence to any other read is identified as a duplicate and removed from the population, thus, only a single copy is retained within the file. Once the duplicate reads have been removed, 8 bases are trimmed from each read. The trimmed reads are aligned to a reference genome. Differential expression is then determined by comparing FPKM (fragments per kilobase per million reads) values between libraries utilizing scripts such as cufflinks or cuffdiff (Trapnell et al. 2010, Nature Biotechnology, 28, 511-515; Trapnell et al. 2013, Nature Biotechnology, 31, 46-53).

Example 6: Sequencing of the Identifier Site With the Target Sequence in Paired End Reads

[00102] The Ovation Library System for Low Complexity Samples (NuGEN Technologies, Inc., San Carlos, CA) was used to generate four libraries, each from a single amplicon, following manufacturer's protocol. Purified libraries were mixed and sequenced as a multiplex on the Illumina MiSeq (Illumina Inc., San Diego, CA) to produce 125nt forward, 8nt index 1, 8nt index 2, and 25nt reverse reads. Because all amplicon reads start and end at the same sequence coordinates, the traditional method of marking library PCR duplicates (marking reads that start and end at the same genomic coordinates as duplicates) cannot be used. Instead, the 0-8nt of random sequence contained in the adaptors ligated to the amplicon were treated as an identifier sequence and used to mark duplicates. Any paired end reads that shared the same length and sequence of these random bases with any other paired end read was called a duplicate. The table below shows the results of this duplicate marking.

Table 1.

Library	Reads	Duplicate Reads	Reads from independent molecules
1	454249	376797	77452
2	439367	364001	75366
3	317760	253057	64703
4	476572	398380	78192

[00103] Table 1 demonstrates the accuracy of the method used to differentiate between duplicate reads and reads from truly independent molecules. The population of reads from independent molecules, depicted in the final column of Table 1, represent sequences from independent amplicon molecules used in generating the libraries.

Example 7: Sequencing of the Identifier Site With the Target Sequence in Reduced Representation Bisulfite (RRBS) Libraries

[00104] Reduced representation bisulfite (RRBS) libraries of the human genome are generated through the complete restriction enzyme digestion of 100ng input sample, followed by selection for short fragments. The resulting pool of fragments are ligated to adaptor sequences comprising an indexing site and an identifier site. The identifier sites comprise either 6 or 8 random nucleotides. The sequences are then sequenced to identify the identifier sites, thus revealing the true number of duplicates in the pool. In the absence of identifier sites, the number of apparent or perceived duplicates are greater than the number of true duplicates. The inclusion of an identifier site results in the identification of the number of true duplicates, as compared to the larger number of apparent or perceived duplicates.

[00105] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes.

[00106] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

[00107] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and

patent application cited herein is not, and should not be taken as an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

[00108] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

CLAIMS

1. A method for detecting a duplicate sequencing read from a population of sample sequencing reads comprising:
 - a) ligating an adaptor to a 5' end of each nucleic acid fragment of a plurality of nucleic acid fragments from one or more samples, wherein the adaptor comprises:
 - (i) an indexing primer binding site;
 - (ii) an indexing site;
 - (iii) an identifier site; and
 - (iv) a target sequence primer binding site;
 - b) amplifying the adapter-nucleic acid fragment ligated products;
 - c) generating a population of sequencing reads from amplified adapter-nucleic acid fragment ligated products; and
 - d) detecting the population of sequencing reads comprising a sequencing read with a duplicate identifier site and target sequence.
2. The method of claim 1, wherein the method further comprises removing from the population of sequence reads the sequencing read with a duplicate identifier site and target sequence.
3. The method of claim 1, wherein the identifier site is sequenced with the indexing site.
4. The method of claim 1, wherein the identifier site is sequenced separately from the indexing site.
5. The method of claim 1, wherein the identifier site is sequenced with the target sequence.
6. The method of claim 1, wherein the identifier site is sequenced separately from the target sequence.
7. The method of claim 1, wherein the adaptor comprises from 5' to 3':

- (i) the indexing primer binding site;
 - (ii) the indexing site;
 - (iii) the identifier site; and
 - (iv) the target sequence primer binding site.
8. The method of claim 1, wherein the adaptor comprises from 5' to 3':
- (i) the indexing primer binding site;
 - (ii) the indexing site;
 - (iii) the target sequence primer binding site; and
 - (iv) the identifier site.
9. The method of claim 1, wherein the plurality of nucleic acid fragments is generated from more than one sample.
10. The method of claim 9, wherein the nucleic acid fragments from each sample has the same indexing site.
11. The method of claim 10, wherein the sequencing reads are separated based on the indexing site.
12. The method of claim 11, wherein the separation of sequencing reads is performed prior to step d).
13. The method of claim 1, wherein the nucleic acid fragments are DNA fragments, RNA fragments, or DNA/RNA fragments.
14. The method of claim 13, wherein the nucleic acid fragments are genomic DNA fragments or cDNA fragments.
15. The method of claim 1, wherein the indexing site is between 2 and 8 nucleotides in length.

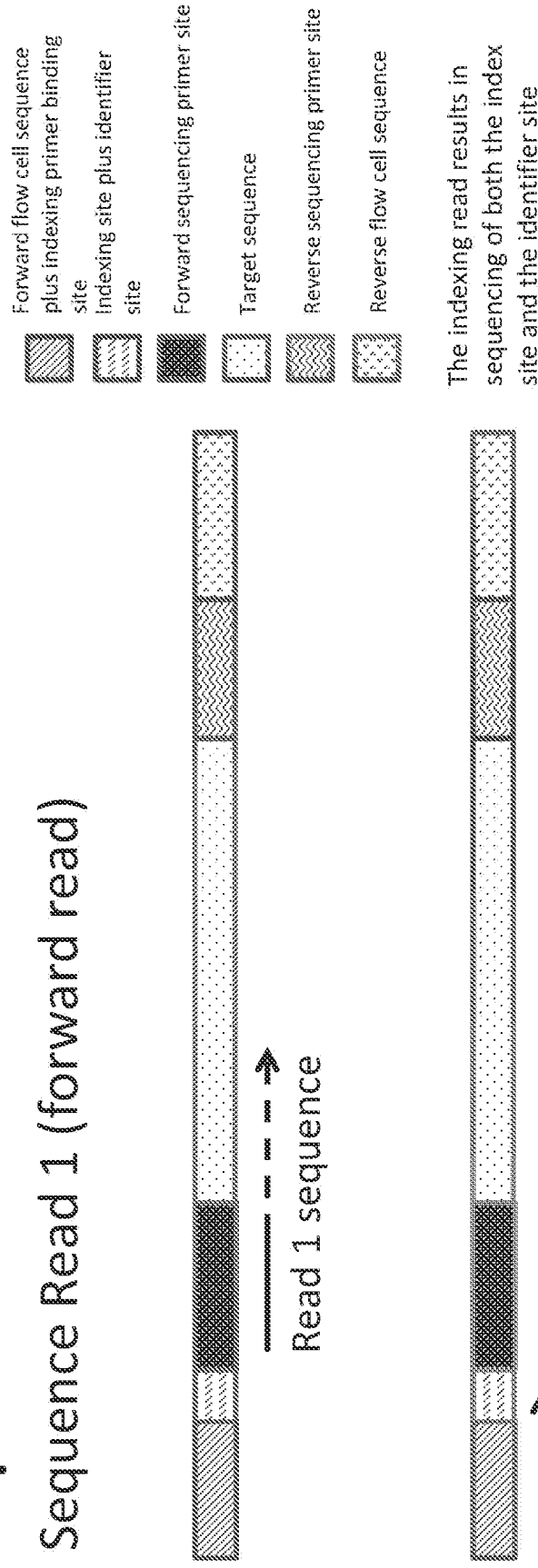
16. The method of claim 1, wherein the indexing site is about 6 nucleotides in length.
17. The method of claim 1, wherein the identifier site is between 1 and 8 nucleotides in length.
18. The method of claim 1, wherein the identifier site is about 8 nucleotides in length.
19. The method of claim 1, wherein the indexing primer binding site is a universal indexing primer binding site.
20. The method of claim 1, wherein the target sequence primer binding site is a universal target sequence primer binding site.
21. A kit comprising a plurality of adaptors, wherein each adaptor comprises:
 - (i) an indexing primer binding site;
 - (ii) an indexing site;
 - (iii) an identifier site; and
 - (iv) a target sequencing primer binding site.

ABSTRACT

The present invention provides methods, compositions and kits for detecting duplicate sequencing reads. In some embodiments, the duplicate sequencing reads are removed.

Standard DNA Sequencing With Duplication Counter

- Sequence Read 1 (forward read)



- Extension of the indexing read beyond the indexing site and into the nucleotides of the identifier site provides data on which nucleic acid molecule is being sequenced.

Figure 1

Single Primer Extension (SPET) Reaction

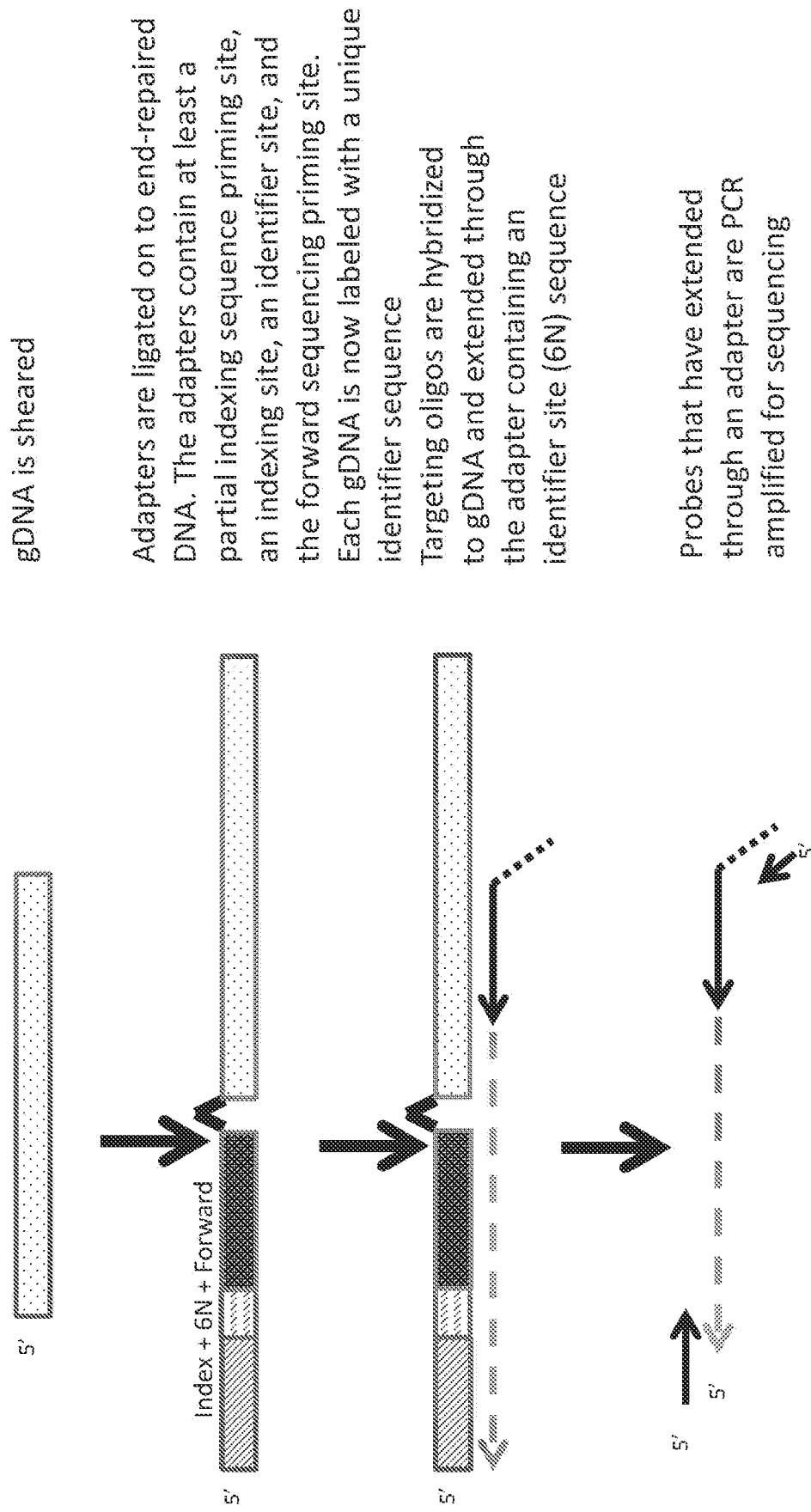
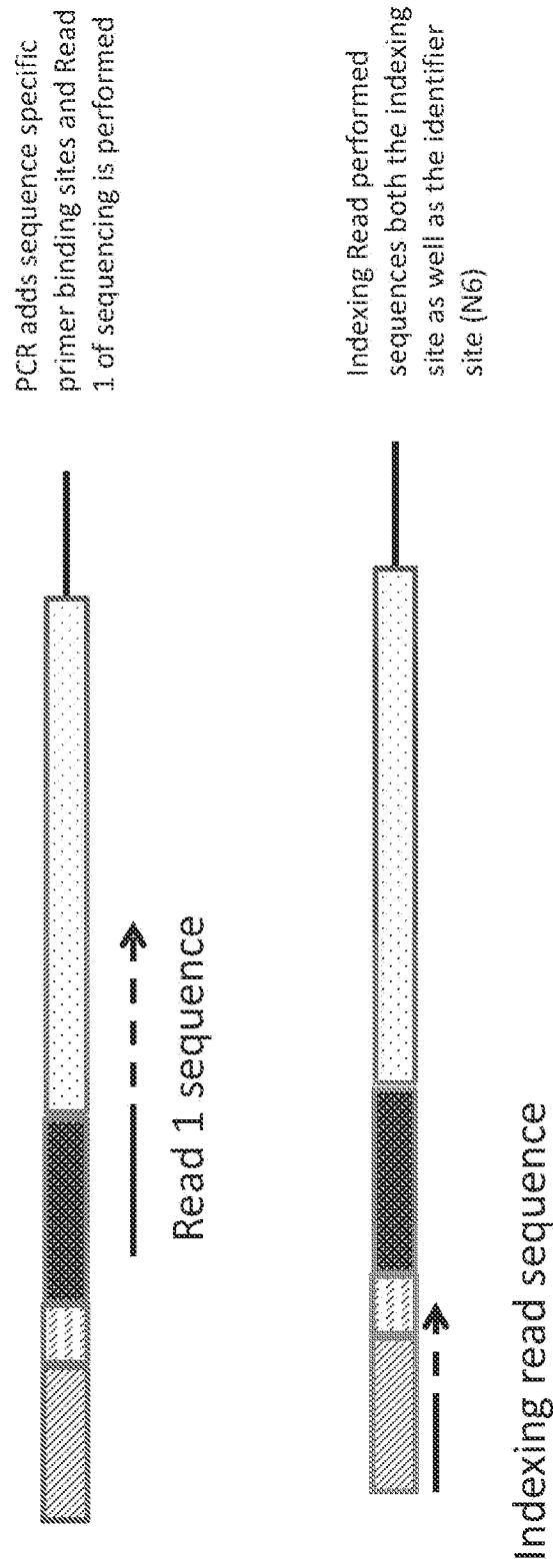


Figure 2A

SPET Sequencing

- Sequence from random end toward the probe



- Extending the indexing read past the 6 base indexing site sequence and into the random Ns of the identifier site provides data on which gDNA molecule the extension reaction occurred upon.

Figure 2B

Example Adaptor

An adaptor sequence with necessary priming sites, the index site, and the identifier site

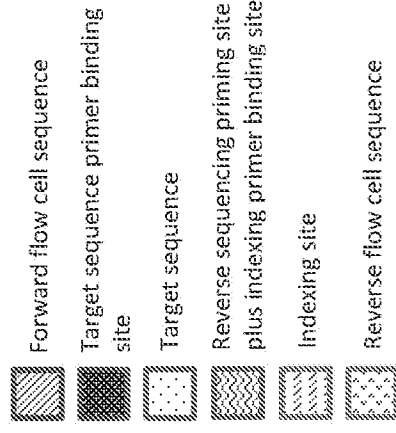
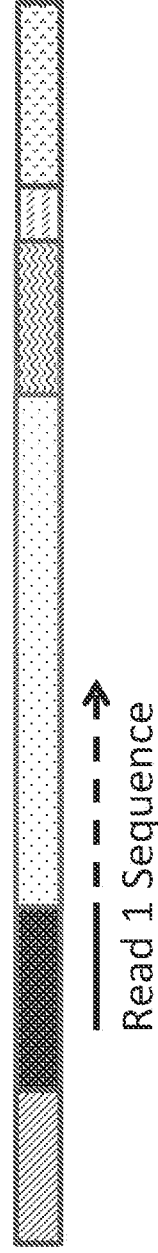
CAGAGCACACGTCTGAACTCCAGTCACAGTGAGNNNNNNACACTTCCCTACACGACGCTCTCCGATCT	(gDNA)

Illumina flow cell plus Indexing read priming site	Indexing site + Identifier site
	Illumina forward sequencing read priming site

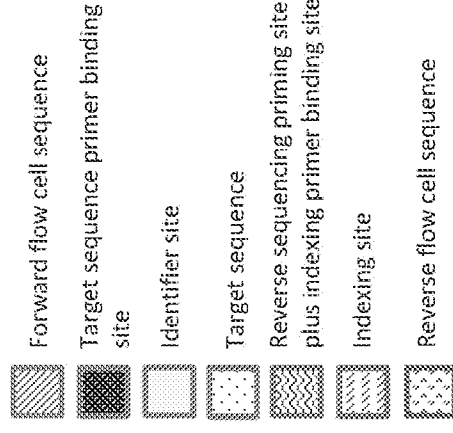
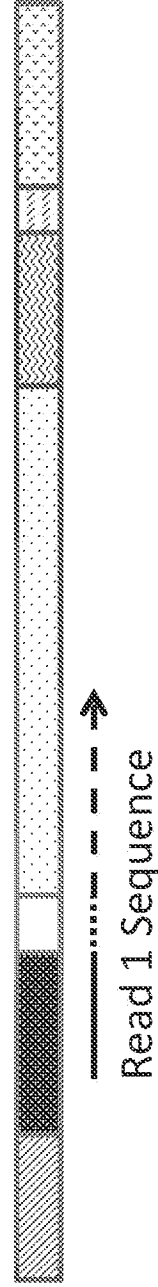
Figure 3

DNA Sequencing With Duplicate Recognition at the insert junction

- Traditional library, sequence read 1 (forward read)



- Duplicate recognition library, sequence read 1 (forward read)



Read 1 initially records the approximately 8 random bases of the identifier site appended to the library sequences, followed by the target sequence

Figure 4

Resolution of apparent duplicate reads by identifier sequence ligation

Libraries were generated from 100ng genomic DNA Covaris fragmented to ~500bp in length. These were enriched with the 344 gene cancer panel, ~12k probes covering 969kb of genomic space and sequenced on the Illumina Miseq. Different numbers of reads were randomly selected for this analysis

Total reads randomly selected	Aligned read	Fold Target Coverage	Total duplicates after identifier analysis (true rate)	Percentage duplicates after identifier analysis (true rate)	Total apparent duplicates with no identifier	Percentage apparent duplicates with no identifier
100K	97,919	2X	1854	1.89	3256	3.33
500K	488,423	7X	39731	8.13	68741	14.07
1M	976,662	13X	145589	14.91	237882	24.36
2M	1,954,673	21X	514848	26.34	764825	39.13
10M	9,771,462	43X	6493577	66.45	7515496	76.91

“Apparent” Duplicates -- individual targets within libraries consist of one random end (result of fragmentation) and one common end (initiated with the sequence specific probe). Apparent duplicates are derived from sequencing libraries and using conventional measures of duplicate reads ie, reads were mapped using bowtie and all reads with the same start and end genomic coordinates were counted as duplicates.

“True” duplicates - An identifier sequence is introduced through ligation to differentiate between fragments of DNA that randomly have the same start and end mapping coordinates. The analysis method is described in the specification.

Figure 5

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of
Invention

COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ

As the below named inventor, I hereby declare that:

This declaration ☐ The attached application, or
is directed to:

☒ United States application or PCT international application number 14/540,917

filed on 11/13/2014.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

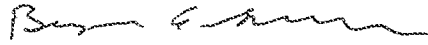
I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: Ben Schroeder Date (Optional) : _____

Signature: 

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. 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 1.11 and 1.14. This collection is estimated to take 1 minute 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.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of
Invention

COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ

As the below named inventor, I hereby declare that:

This declaration
is directed to:

☐

The attached application, or

☒

United States application or PCT international application number 14/540,917

filed on 11/13/2014.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: Douglas Amorese

Date (Optional) : _____

Signature: _____

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of
Invention

COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ

As the below named inventor, I hereby declare that:

This declaration ☐ The attached application, or
is directed to:

☒ United States application or PCT international application number 14/540,917

filed on 11/13/2014.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

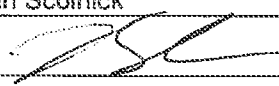
I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

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LEGAL NAME OF INVENTOR

Inventor: Jonathan Scolnick Date (Optional): _____

Signature: 

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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