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UTILITY PATENT APPLICATION TRANSMITTAL <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%;">Attorney Docket No.</td> <td>104371-5016-US10</td> </tr> <tr> <td>First Named Inventor</td> <td>Sydney Brenner</td> </tr> <tr> <td>Title</td> <td>METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS</td> </tr> <tr> <td>Priority Mail Express® Label No.</td> <td></td> </tr> </table>	Attorney Docket No.	104371-5016-US10	First Named Inventor	Sydney Brenner	Title	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS	Priority Mail Express® Label No.	
Attorney Docket No.	104371-5016-US10								
First Named Inventor	Sydney Brenner								
Title	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS								
Priority Mail Express® Label No.									

APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents.</i>	ADDRESS TO: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450
1. ☐ Fee Transmittal Form (PTO/SB/17 or equivalent) 2. ☒ Applicant asserts small entity status. See 37 CFR 1.27 3. ☐ Applicant certifies micro entity status. See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent. 4. ☒ Specification [Total Pages <u>44</u>] Both the claims and abstract must start on a new page. (See MPEP § 608.01(a) for information on the preferred arrangement) 5. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets <u>13</u>] 6. Inventor's Oath or Declaration [Total Pages <u>4</u>] (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e)) a. ☐ Newly executed (original or copy) b. ☒ A copy from a prior application (37 CFR 1.63(d)) 7. ☒ Application Data Sheet * See note below. See 37 CFR 1.76 (PTO/AIA/14 or equivalent) 8. CD-ROM or CD-R in duplicate, large table, or Computer Program (Appendix) ☐ Landscape Table on CD 9. Nucleotide and/or Amino Acid Sequence Submission (if applicable, items a. – c. are required) a. ☐ Computer Readable Form (CRF) b. ☐ Specification Sequence Listing on: i. ☐ CD-ROM or CD-R (2 copies); or ii. ☐ Paper c. ☐ Statements verifying identity of above copies	ACCOMPANYING APPLICATION PAPERS 10. ☐ Assignment Papers (cover sheet & document(s)) Name of Assignee _____ 11. ☐ 37 CFR 3.73(c) Statement ☐ Power of Attorney (when there is an assignee) 12. ☐ English Translation Document (if applicable) 13. ☐ Information Disclosure Statement (PTO/SB/08 or PTO-1449) ☐ Copies of citations attached 14. ☐ Preliminary Amendment 15. ☐ Return Receipt Postcard (MPEP § 503) (Should be specifically itemized) 16. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed) 17. ☐ Nonpublication Request Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent. 18. ☐ Other: _____ _____ _____ _____

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
 (2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS					
☒ The address associated with Customer Number: <u>156830</u> OR ☐ Correspondence address below					
Name					
Address					
City	State		Zip Code		
Country	Telephone		Email		
Signature	/Gargi Talukder/		Date	November 16, 2018	
Name (Print/Type)	Gargi Talukder		Registration No. (Attorney/Agent)	61,368	

This collection of information is required by 37 CFR 1.53(b). 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 12 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, 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.

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 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.

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	104371-5016-US10
		Application Number	
Title of Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		
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	
	Sydney		Brenner		
Residence Information (Select One) US Residency • Non US Residency Active US Military Service					
City	Ely	Country of Residence ⁱ	GB		
Mailing Address of Inventor:					
Address 1		3 Barton Square			
Address 2					
City	Ely	State/Province			
Postal Code	CB7 4PJ	Country ⁱ	GB		
Inventor	2				Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Gi		Mikawa		
Residence Information (Select One) US Residency • Non US Residency Active US Military Service					
City	Great Shelford	Country of Residence ⁱ	GB		
Mailing Address of Inventor:					
Address 1		104 High Street			
Address 2					
City	Great Shelford	State/Province			
Postal Code	CB1 6A3	Country ⁱ	GB		
Inventor	3				Remove
Legal Name					

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	104371-5016-US10
		Application Number	
Title of Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		

Prefix	Given Name	Middle Name	Family Name	Suffix
	Robert		Osborne	
Residence Information (Select One) US Residency ☒ Non US Residency Active US Military Service				
City	Great Chesterford	Country of Residence ⁱ	GB	

Mailing Address of Inventor:

Address 1	38 Pilgrim Close			
Address 2				
City	Great Chesterford	State/Province		
Postal Code	CB10 1QB	Country ⁱ	GB	
Inventor	4	Remove		
Legal Name				

Prefix	Given Name	Middle Name	Family Name	Suffix
	Andrew		Slatter	
Residence Information (Select One) US Residency ☒ Non US Residency Active US Military Service				
City	London	Country of Residence ⁱ	GB	

Mailing Address of Inventor:

Address 1	65a Huddleston Road			
Address 2				
City	London	State/Province		
Postal Code	N7 0AE	Country ⁱ	GB	
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	156830		
Email Address	sfipdocketing@morganlewis.com	Add Email	Remove Email

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	104371-5016-US10
		Application Number	
Title of Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		

Application Information:

Title of the Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		
Attorney Docket Number	104371-5016-US10	Small Entity Status Claimed	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	13	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.

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	156830		

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	104371-5016-US10
		Application Number	
Title of Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		

Domestic Benefit/National Stage Information:

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	15/677957	2017-08-15		
Prior Application Status	Abandoned	Remove			
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
15/677957	Continuation of	14/792094	2015-07-06		
Prior Application Status	Patented	Remove			
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
14/792094	Continuation of	14/172694	2014-02-04	9102980	2015-08-11
Prior Application Status	Patented	Remove			
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
14/172694	Continuation of	14/021790	2013-09-09	8679756	2014-03-25
Prior Application Status	Patented	Remove			
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
14/021790	Continuation of	13/859450	2013-04-09	8563274	2013-10-22
Prior Application Status	Abandoned	Remove			
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
13/859450	Continuation of	13/622872	2012-09-19		
Prior Application Status	Patented	Remove			
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
13/622872	Continuation of	13/387343	2012-02-15	8298767	2012-10-30
Prior Application Status	Expired	Remove			
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
13/387343	a 371 of international	PCTIB2010002243	2010-08-13		

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	104371-5016-US10	
		Application Number		
Title of Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS			

Prior Application Status	Expired	Remove		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)	
PCTIB2010002243	Claims benefit of provisional	61/288792	2009-12-21	
Prior Application Status	Expired	Remove		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)	
PCTIB2010002243	Claims benefit of provisional	61/235595	2009-08-20	
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.				Add

Foreign Priority Information:

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).

			Remove
Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			Add

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	104371-5016-US10
		Application Number	
Title of Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		

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.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

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.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ 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.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	104371-5016-US10
		Application Number	
Title of Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		

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	10X Genomics, Inc.		
Mailing Address Information For Applicant:			
Address 1	7068 Koll Center Parkway, Suite 401		
Address 2			
City	Pleasanton	State/Province	CA
Country	US	Postal Code	94566
Phone Number		Fax Number	
Email Address			
Additional Applicant Data may be generated within this form by selecting the Add button. Add			

Assignee Information including Non-Applicant Assignee 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.

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	104371-5016-US10
		Application Number	
Title of Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		

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.				
				Remove
If the Assignee or Non-Applicant Assignee is an Organization check here. ☐				
Prefix	Given Name	Middle Name	Family Name	Suffix
Mailing Address Information For Assignee including Non-Applicant Assignee:				
Address 1				
Address 2				
City		State/Province		
Country ⁱ		Postal Code		
Phone Number		Fax Number		
Email Address				
Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.				Add

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the **INITIAL** filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Gargi Talukder/		Date (YYYY-MM-DD)	2018-11-16
First Name	Gargi	Last Name	Talukder	Registration Number
				61368
Additional Signature may be generated within this form by selecting the Add button.				Add

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	104371-5016-US10
		Application Number	
Title of Invention	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		

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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.

ABSTRACT OF THE DISCLOSURE

Aspects of the present invention include analyzing nucleic acids from single cells using methods that include using tagged polynucleotides containing multiplex identifier sequences.

CLAIMS

What is claimed:

1. A method of counting nucleic acids in a sample, the method comprising:
 - (a) providing a sample comprising a plurality of cells, wherein a cell of the plurality of cells comprises a plurality of sample polynucleotides;
 - (b) generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides of said cell, wherein a tagged polynucleotide of the plurality of tagged polynucleotides comprises:
 - (i) a sequence from a sample polynucleotide of the plurality of sample polynucleotides;
 - (ii) a first tag sequence distinguishing said sample polynucleotide from sample polynucleotides from other cells; and
 - (iii) a second tag sequence distinguishing said sample polynucleotide from other sample polynucleotides from said cell;
 - (c) sequencing the tagged polynucleotide to determine the sequence from said sample polynucleotide, the first tag sequence, and the second tag sequence;
 - (d) using the first tag sequence and the second tag sequence to count a number of sample polynucleotides in said plurality of sample polynucleotides of said cell having said sequence from said sample polynucleotide.
2. The method of claim 1, wherein the method further comprises amplifying the plurality of tagged polynucleotides prior to the sequencing step (c).
3. The method of claim 1, wherein the plurality of sample polynucleotides is selected from DNA and RNA.
4. The method of claim 3, wherein the plurality of sample polynucleotides comprises mRNA.
5. The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one ligation reaction.
6. The method of claim 1, wherein the plurality of tagged polynucleotides is generated through amplification of the plurality of sample polynucleotides using an adapter sequence comprising an amplification primer, the first tag sequence, and the second tag sequence.

7. The method of claim 1, wherein the plurality of tagged polynucleotides is generated through a polymerase chain reaction (PCR) on the plurality of sample polynucleotides using an adapter sequence comprising an amplification primer, the first tag sequence, and the second tag sequence.
8. The method of claim 1, wherein the plurality of tagged polynucleotides is generated through linear amplification of the plurality of sample polynucleotides using an adapter sequence comprising an amplification primer, the first tag sequence, and the second tag sequence.
9. The method of claim 1, wherein the method further comprises amplifying the tagged polynucleotide of the plurality of tagged polynucleotides prior to the sequencing step (c).
10. The method of claim 1, wherein the sample polynucleotide of the plurality of sample polynucleotides is selected from DNA and RNA.
11. The method of claim 10, wherein the sample polynucleotide of the plurality of sample polynucleotides comprises mRNA.
12. The method of claim 1, wherein the tagged polynucleotide of the plurality of tagged polynucleotides is generated through at least one ligation reaction.
13. The method of claim 1, wherein the tagged polynucleotide of the plurality of tagged polynucleotides is generated through amplification of the sample polynucleotide of the plurality of sample polynucleotides using an adapter sequence comprising an amplification primer, the first tag sequence, and the second tag sequence.
14. The method of claim 1, wherein the tagged polynucleotide of the plurality of tagged polynucleotides is generated through a polymerase chain reaction (PCR) on the sample polynucleotide of the plurality of sample polynucleotides using an adapter sequence comprising an amplification primer, the first tag sequence, and the second tag sequence.

15. The method of claim 1, wherein the tagged polynucleotide of the plurality of tagged polynucleotides is generated through linear amplification of the sample polynucleotide of the plurality of sample polynucleotides using an adapter sequence comprising an amplification primer, the first tag sequence, and the second tag sequence.

METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

BACKGROUND OF THE INVENTION

[0001] We have previously described methods that enable tagging each of a population of fragmented genomes and then combining them together to create a ‘population library’ that can be processed and eventually sequenced as a mixture. The population tags enable analysis software to parse the sequence reads into files that can be attributed to a particular genome in the population. One limitation of the overall process stems from limitations of existing DNA sequencing technologies. In particular, if fragments in the regions of interest of the genome are longer than the lengths that can be sequenced by a particular technology, then such fragments will not be fully analyzed (since sequencing proceeds from an end of a fragment inward). Furthermore, a disadvantage of any sequencing technology dependent on fragmentation is that sequence changes in one part of a particular genomic region may not be able to be linked to sequence changes in other parts of the same genome (e.g., the same chromosome) because the sequence changes reside on different fragments. (See Figure 5 and its description below).

[0002] The present invention removes the limitations imposed by current sequencing technologies as well as being useful in a number of other nucleic acid analyses.

SUMMARY OF THE INVENTION

[0003] Aspects of the present invention are drawn to processes for moving a region of interest in a polynucleotide from a first position to a second position with regard to a domain within the polynucleotide, also referred to as a “reflex method” (or reflex process, reflex sequence process, reflex reaction, and the like). In certain embodiments, the reflex method results in moving a region of interest into functional proximity to specific domain elements present in the polynucleotide (e.g., primer sites and/or MID). Compositions, kits and systems that find use in carrying out the reflex processes described herein are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to scale. Indeed, the

dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

[0005] Figure 1: Panel A is a schematic diagram illustrating moving a first domain from one site to another in a nucleic acid molecule using a reflex sequence. Panel B is a schematic diagram depicting the relative position of primer pairs (A_n - B_n primers) that find use in aspects of the reflex process described herein.

[0006] Figure 2 shows an exemplary embodiment of using binding partner pairs (biotin/streptavidin) to isolate single stranded polynucleotides of interest.

[0007] Figure 3 is a schematic diagram illustrating an exemplary embodiment for moving a primer site and a MID to a specific location in a nucleic acid of interest.

[0008] Figure 4 shows a schematic diagram illustrating an exemplary use of the reflex process for generating a sample enriched for fragments having a region of interest (e.g., from a population of randomly fragmented and asymmetrically tagged polynucleotides).

[0009] Figure 5 shows a comparison of methods for identifying nucleic acid polymorphisms in homologous nucleic acids in a sample (e.g., the same region derived from a chromosomal pair of a diploid cell or viral genomes/transcripts). The top schematic shows two nucleic acid molecules in a sample (1 and 2) having a different assortment of polymorphisms in polymorphic sites A, B and C (A_1 , B_1 , C_1 and C_2). Standard sequencing methods using fragmentation (left side) can identify the polymorphisms in these nucleic acids but do not retain linkage information. Employing the reflex process described herein to identify polymorphisms (right side) maintains linkage information.

[0010] Figure 6: Panel A is a schematic showing expected structures and sizes of nucleic acid species in the reflex process; Panel B is a polyacrylamide gel showing the nucleic acid species produced in the reflex process described in Example 1.

[0011] Figure 7: Panel A is a schematic showing the structure of the nucleic acid and competitor used in the reflex process; Panel B is a polyacrylamide gel showing the nucleic acid species produced in the reflex process described in Example 1.

[0012] Figure 8 shows a flow chart of a reflex process (left) in which the T7 exonuclease step is optional. The gel on the right shows the resultant product of the reflex process either without the T7 exonuclease step (lane 1) or with the T7 exonuclease step (lane 2).

[0013] Figure 9 shows an exemplary reflex process workflow with indications on the right as to where purification of reaction products is employed (e.g., using Agencourt beads to remove primer oligos).

[0014] Figure 10 shows the starting material (left panel) and the resultant product generated (right panel) using a reflex process without using a T7 exonuclease step (as described in Example II). The reflex site in the starting material is a sequence normally present in the polynucleotide being processed (also called a “non-artificial” reflex site). This figure shows that the 755 base pair starting nucleic acid was processed to the expected 461 base pair product, thus confirming that a “non-artificial” reflex site is effective in transferring an adapter domain from one location to another in a polynucleotide of interest in a sequence specific manner.

[0015] Figure 11 shows a schematic and results of an experiment in which the reflex process is performed on a single large initial template (a “parent” fragment) to generate five different products (“daughter” products) each having a different region of interest (i.e., daughter products are produced having either region 1, 2, 3, 4 or 5).

[0016] Figure 12 shows a schematic and results of experiments performed to determine the prevalence of intramolecular rearrangement during the reflex process (as desired) vs. intermolecular rearrangement (MID switching).

[0017] Figure 13 shows a diagram of exemplary workflows for preparing material for and performing the reflex process.

DEFINITIONS

[0018] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Still, certain elements are defined for the sake of clarity and ease of reference.

[0019] Terms and symbols of nucleic acid chemistry, biochemistry, genetics, and molecular biology 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.

[0020] “Amplicon” means the product of a polynucleotide amplification reaction. That is, it is a population of polynucleotides, usually double stranded, that are replicated from one or

more starting sequences. The one or more starting sequences may be one or more copies of the same sequence, or it may be a mixture of different sequences. Amplicons may be produced by a variety of amplification reactions whose products are multiple replicates of one or more target nucleic acids. Generally, amplification reactions producing amplicons are “template-driven” in that base pairing of reactants, either nucleotides or oligonucleotides, have complements in a template polynucleotide that are required for the creation of reaction products. In one aspect, template-driven reactions are primer extensions with a nucleic acid polymerase or oligonucleotide ligations with a nucleic acid ligase. Such reactions include, but are not limited to, polymerase chain reactions (PCRs), linear polymerase reactions, nucleic acid sequence-based amplification (NASBAs), rolling circle amplifications, and the like, disclosed in the following references that are incorporated herein by reference: Mullis et al, U.S. patents 4,683,195; 4,965,188; 4,683,202; 4,800,159 (PCR); Gelfand et al, U.S. patent 5,210,015 (real-time PCR with “TAQMAN™” probes); Wittwer et al, U.S. patent 6,174,670; Kacian et al, U.S. patent 5,399,491 (“NASBA”); Lizardi, U.S. patent 5,854,033; Aono et al, Japanese patent publ. JP 4-262799 (rolling circle amplification); and the like. In one aspect, amplicons of the invention are produced by PCRs. An amplification reaction may be a “real-time” amplification if a detection chemistry is available that permits a reaction product to be measured as the amplification reaction progresses, e.g. “real-time PCR” described below, or “real-time NASBA” as described in Leone et al, *Nucleic Acids Research*, 26: 2150-2155 (1998), and like references. As used herein, the term “amplifying” means performing an amplification reaction. A “reaction mixture” means a solution containing all the necessary reactants for performing a reaction, which may include, but not be limited to, buffering agents to maintain pH at a selected level during a reaction, salts, co-factors, scavengers, and the like.

[0021] The term “assessing” includes any form of measurement, and includes determining if an element is present or not. The terms “determining”, “measuring”, “evaluating”, “assessing” and “assaying” are used interchangeably and includes quantitative and qualitative determinations. Assessing may be relative or absolute. “Assessing the presence of” includes determining the amount of something present, and/or determining whether it is present or absent. As used herein, the terms “determining,” “measuring,” and “assessing,” and “assaying” are used interchangeably and include both quantitative and qualitative determinations.

[0022] Polynucleotides that are “asymmetrically tagged” have left and right adapter domains that are not identical. This process is referred to generically as attaching adapters asymmetrically or asymmetrically tagging a polynucleotide, e.g., a polynucleotide fragment. Production of polynucleotides having asymmetric adapter termini may be achieved in any convenient manner. Exemplary asymmetric adapters are described in: U.S. Patents 5,712,126 and 6,372,434; U.S. Patent Publications 2007/0128624 and 2007/0172839; and PCT publication WO/2009/032167; all of which are incorporated by reference herein in their entirety. In certain embodiments, the asymmetric adapters employed are those described in U.S. Patent Application Ser. No. 12/432,080, filed on April 29, 2009, incorporated herein by reference in its entirety.

[0023] As one example, a user of the subject invention may use an asymmetric adapter to tag polynucleotides. An “asymmetric adapter” is one that, when ligated to both ends of a double stranded nucleic acid fragment, will lead to the production of primer extension or amplification products that have non-identical sequences flanking the genomic insert of interest. The ligation is usually followed by subsequent processing steps so as to generate the non-identical terminal adapter sequences. For example, replication of an asymmetric adapter attached fragment(s) results in polynucleotide products in which there is at least one nucleic acid sequence difference, or nucleotide/nucleoside modification, between the terminal adapter sequences. Attaching adapters asymmetrically to polynucleotides (e.g., polynucleotide fragments) results in polynucleotides that have one or more adapter sequences on one end (e.g., one or more region or domain, e.g., a primer site) that are either not present or have a different nucleic acid sequence as compared to the adapter sequence on the other end. It is noted that an adapter that is termed an “asymmetric adapter” is not necessarily itself structurally asymmetric, nor does the mere act of attaching an asymmetric adapter to a polynucleotide fragment render it immediately asymmetric. Rather, an asymmetric adapter-attached polynucleotide, which has an identical asymmetric adapter at each end, produces replication products (or isolated single stranded polynucleotides) that are asymmetric with respect to the adapter sequences on opposite ends (e.g., after at least one round of amplification/primer extension).

[0024] Any convenient asymmetric adapter, or process for attaching adapters asymmetrically, may be employed in practicing the present invention. Exemplary asymmetric adapters are described in: U.S. Patents 5,712,126 and 6,372,434; U.S. Patent Publications 2007/0128624 and 2007/0172839; and PCT publication WO/2009/032167; all

of which are incorporated by reference herein in their entirety. In certain embodiments, the asymmetric adapters employed are those described in U.S. Patent Application Ser. No. 12/432,080, filed on April 29, 2009, incorporated herein by reference in its entirety.

[0025] "Complementary" or "substantially complementary" refers to the hybridization or base pairing or the formation of a duplex between nucleotides or nucleic acids, such as, for instance, between the two strands of a double stranded DNA molecule or between an oligonucleotide primer and a primer site on a single stranded nucleic acid. Complementary nucleotides are, generally, A and T (or A and U), or C and G. Two single stranded RNA or DNA molecules are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 80% of the nucleotides of the other strand, usually at least about 90% to 95%, and more preferably from about 98 to 100%. Alternatively, substantial complementarity exists when an RNA or DNA strand will hybridize under selective hybridization conditions to its complement. Typically, selective hybridization will occur when there is at least about 65% complementary over a stretch of at least 14 to 25 nucleotides, preferably at least about 75%, more preferably at least about 90% complementary. See, M. Kanehisa Nucleic Acids Res. 12:203 (1984), incorporated herein by reference.

[0026] "Duplex" means at least two oligonucleotides and/or polynucleotides that are fully or partially complementary undergo Watson-Crick type base pairing among all or most of their nucleotides so that a stable complex is formed. The terms "annealing" and "hybridization" are used interchangeably to mean the formation of a stable duplex. "Perfectly matched" in reference to a duplex means that the poly- or oligonucleotide strands making up the duplex form a double stranded structure with one another such that every nucleotide in each strand undergoes Watson-Crick base pairing with a nucleotide in the other strand. A stable duplex can include Watson-Crick base pairing and/or non-Watson-Crick base pairing between the strands of the duplex (where base pairing means the forming hydrogen bonds). In certain embodiments, a non-Watson-Crick base pair includes a nucleoside analog, such as deoxyinosine, 2, 6-diaminopurine, PNAs, LNA's and the like. In certain embodiments, a non-Watson-Crick base pair includes a "wobble base", such as deoxyinosine, 8-oxo-dA, 8-oxo-dG and the like, where by "wobble base" is meant a nucleic acid base that can base pair with a first nucleotide base in a complementary nucleic acid strand but that, when employed as a template strand for nucleic acid synthesis, leads to the

incorporation of a second, different nucleotide base into the synthesizing strand (wobble bases are described in further detail below). A "mismatch" in a duplex between two oligonucleotides or polynucleotides means that a pair of nucleotides in the duplex fails to undergo Watson-Crick bonding.

[0027] "Genetic locus," "locus," or "locus of interest" in reference to a genome or target polynucleotide, means a contiguous sub-region or segment of the genome or target polynucleotide. As used herein, genetic locus, locus, or locus of interest may refer to the position of a nucleotide, a gene or a portion of a gene in a genome, including mitochondrial DNA or other non-chromosomal DNA (e.g., bacterial plasmid), or it may refer to any contiguous portion of genomic sequence whether or not it is within, or associated with, a gene. A genetic locus, locus, or locus of interest can be from a single nucleotide to a segment of a few hundred or a few thousand nucleotides in length or more. In general, a locus of interest will have a reference sequence associated with it (see description of "reference sequence" below).

[0028] "Kit" refers to any delivery system for delivering materials or reagents for carrying out a method of the invention. In the context of reaction assays, such delivery systems include systems that allow for the storage, transport, or delivery of reaction reagents (e.g., probes, enzymes, etc. in the appropriate containers) and/or supporting materials (e.g., buffers, written instructions for performing the assay etc.) from one location to another. For example, kits include one or more enclosures (e.g., boxes) containing the relevant reaction reagents and/or supporting materials. Such contents may be delivered to the intended recipient together or separately. For example, a first container may contain an enzyme for use in an assay, while a second container contains probes.

[0029] "Ligation" means to form a covalent bond or linkage between the termini of two or more nucleic acids, e.g. oligonucleotides and/or polynucleotides, in a template-driven reaction. The nature of the bond or linkage may vary widely and the ligation may be carried out enzymatically or chemically. As used herein, ligations are usually carried out enzymatically to form a phosphodiester linkage between a 5' carbon of a terminal nucleotide of one oligonucleotide with 3' carbon of another oligonucleotide. A variety of template-driven ligation reactions are described in the following references, which are incorporated by reference: Whiteley et al, U.S. patent 4,883,750; Letsinger et al, U.S. patent 5,476,930; Fung et al, U.S. patent 5,593,826; Kool, U.S. patent 5,426,180; Landegren et al, U.S. patent 5,871,921; Xu and Kool, *Nucleic Acids Research*, 27: 875-881 (1999); Higgins et al,

Methods in Enzymology, 68: 50-71 (1979); Engler et al, The Enzymes, 15: 3-29 (1982); and Namsaraev, U.S. patent publication 2004/0110213.

[0030] “Multiplex Identifier” (MID) as used herein refers to a tag or combination of tags associated with a polynucleotide whose identity (e.g., the tag DNA sequence) can be used to differentiate polynucleotides in a sample. In certain embodiments, the MID on a polynucleotide is used to identify the source from which the polynucleotide is derived. For example, a nucleic acid sample may be a pool of polynucleotides derived from different sources, (e.g., polynucleotides derived from different individuals, different tissues or cells, or polynucleotides isolated at different times points), where the polynucleotides from each different source are tagged with a unique MID. As such, a MID provides a correlation between a polynucleotide and its source. In certain embodiments, MIDs are employed to uniquely tag each individual polynucleotide in a sample. Identification of the number of unique MIDs in a sample can provide a readout of how many individual polynucleotides are present in the sample (or from how many original polynucleotides a manipulated polynucleotide sample was derived; see, e.g., U.S. Patent No. 7,537,897, issued on May 26, 2009, incorporated herein by reference in its entirety). MIDs can range in length from 2 to 100 nucleotide bases or more and may include multiple subunits, where each different MID has a distinct identity and/or order of subunits. Exemplary nucleic acid tags that find use as MIDs are described in U.S. Patent 7,544,473, issued on June 6, 2009, and titled “Nucleic Acid Analysis Using Sequence Tokens”, as well as U.S. Patent 7,393,665, issued on July 1, 2008, and titled “Methods and Compositions for Tagging and Identifying Polynucleotides”, both of which are incorporated herein by reference in their entirety for their description of nucleic acid tags and their use in identifying polynucleotides. In certain embodiments, a set of MIDs employed to tag a plurality of samples need not have any particular common property (e.g., T_m, length, base composition, etc.), as the methods described herein can accommodate a wide variety of unique MID sets. It is emphasized here that MIDs need only be unique within a given experiment. Thus, the same MID may be used to tag a different sample being processed in a different experiment. In addition, in certain experiments, a user may use the same MID to tag a subset of different samples within the same experiment. For example, all samples derived from individuals having a specific phenotype may be tagged with the same MID, e.g., all samples derived from control (or wildtype) subjects can be tagged with a first MID while subjects having a disease condition can be tagged with a second MID (different than the first MID). As another example, it may be desirable to tag

different samples derived from the same source with different MIDs (e.g., samples derived over time or derived from different sites within a tissue). Further, MIDs can be generated in a variety of different ways, e.g., by a combinatorial tagging approach in which one MID is attached by ligation and a second MID is attached by primer extension. Thus, MIDs can be designed and implemented in a variety of different ways to track polynucleotide fragments during processing and analysis, and thus no limitation in this regard is intended.

[0031] "Nucleoside" as used herein includes the natural nucleosides, including 2'-deoxy and 2'-hydroxyl forms, e.g. as described in Kornberg and Baker, *DNA Replication*, 2nd Ed. (Freeman, San Francisco, 1992). "Analog" in reference to nucleosides includes synthetic nucleosides having modified base moieties and/or modified sugar moieties, e.g. described by Scheit, *Nucleotide Analogs* (John Wiley, New York, 1980); Uhlman and Peyman, *Chemical Reviews*, 90: 543-584 (1990), or the like, with the proviso that they are capable of specific hybridization. Such analogs include synthetic nucleosides designed to enhance binding properties, reduce complexity, increase specificity, and the like. Polynucleotides comprising analogs with enhanced hybridization or nuclease resistance properties are described in Uhlman and Peyman (cited above); Crooke et al, *Exp. Opin. Ther. Patents*, 6: 855-870 (1996); Mesmaeker et al, *Current Opinion in Structural Biology*, 5: 343-355 (1995); and the like. Exemplary types of polynucleotides that are capable of enhancing duplex stability include oligonucleotide N3'→P5' phosphoramidates (referred to herein as "amidates"), peptide nucleic acids (referred to herein as "PNAs"), oligo-2'-O-alkylribonucleotides, polynucleotides containing C-5 propynylpyrimidines, locked nucleic acids ("LNAs"), and like compounds. Such oligonucleotides are either available commercially or may be synthesized using methods described in the literature.

[0032] "Polymerase chain reaction," or "PCR," means a reaction for the in vitro amplification of specific DNA sequences by the simultaneous primer extension of complementary strands of DNA. In other words, PCR is a reaction for making multiple copies or replicates of a target nucleic acid flanked by primer sites, such reaction comprising one or more repetitions of the following steps: (i) denaturing the target nucleic acid, (ii) annealing primers to the primer sites, and (iii) extending the primers by a nucleic acid polymerase in the presence of nucleoside triphosphates. Usually, the reaction is cycled through different temperatures optimized for each step in a thermal cycler instrument. Particular temperatures, durations at each step, and rates of change between steps depend on many factors well-known to those of ordinary skill in the art, e.g. exemplified by the

references: McPherson et al, editors, PCR: A Practical Approach and PCR2: A Practical Approach (IRL Press, Oxford, 1991 and 1995, respectively). For example, in a conventional PCR using Taq DNA polymerase, a double stranded target nucleic acid may be denatured at a temperature $>90^{\circ}\text{C}$, primers annealed at a temperature in the range $50\text{-}75^{\circ}\text{C}$, and primers extended at a temperature in the range $72\text{-}78^{\circ}\text{C}$. The term “PCR” encompasses derivative forms of the reaction, including but not limited to, RT-PCR, real-time PCR, nested PCR, quantitative PCR, multiplexed PCR, and the like. Reaction volumes range from a few hundred nanoliters, e.g. 200 nL, to a few hundred μL , e.g. 200 μL . “Reverse transcription PCR,” or “RT-PCR,” means a PCR that is preceded by a reverse transcription reaction that converts a target RNA to a complementary single stranded DNA, which is then amplified, e.g. Tecott et al, U.S. patent 5,168,038, which patent is incorporated herein by reference. “Real-time PCR” means a PCR for which the amount of reaction product, i.e. amplicon, is monitored as the reaction proceeds. There are many forms of real-time PCR that differ mainly in the detection chemistries used for monitoring the reaction product, e.g. Gelfand et al, U.S. patent 5,210,015 (“TAQMANTM”); Wittwer et al, U.S. patents 6,174,670 and 6,569,627 (intercalating dyes); Tyagi et al, U.S. patent 5,925,517 (molecular beacons); which patents are incorporated herein by reference. Detection chemistries for real-time PCR are reviewed in Mackay et al, Nucleic Acids Research, 30: 1292-1305 (2002), which is also incorporated herein by reference. “Nested PCR” means a two-stage PCR wherein the amplicon of a first PCR becomes the sample for a second PCR using a new set of primers, at least one of which binds to an interior location of the first amplicon. As used herein, “initial primers” in reference to a nested amplification reaction mean the primers used to generate a first amplicon, and “secondary primers” mean the one or more primers used to generate a second, or nested, amplicon. “Multiplexed PCR” means a PCR wherein multiple target sequences (or a single target sequence and one or more reference sequences) are simultaneously carried out in the same reaction mixture, e.g. Bernard et al, Anal. Biochem., 273: 221-228 (1999)(two-color real-time PCR). Usually, distinct sets of primers are employed for each sequence being amplified.

[0033] “Quantitative PCR” means a PCR designed to measure the abundance of one or more specific target sequences in a sample or specimen. Quantitative PCR includes both absolute quantitation and relative quantitation of such target sequences. Quantitative measurements are made using one or more reference sequences that may be assayed separately or together with a target sequence. The reference sequence may be endogenous or

exogenous to a sample or specimen, and in the latter case, may comprise one or more competitor templates. Typical endogenous reference sequences include segments of transcripts of the following genes: β -actin, GAPDH, β_2 -microglobulin, ribosomal RNA, and the like. Techniques for quantitative PCR are well-known to those of ordinary skill in the art, as exemplified in the following references that are incorporated by reference: Freeman et al, *Biotechniques*, 26: 112-126 (1999); Becker-Andre et al, *Nucleic Acids Research*, 17: 9437-9447 (1989); Zimmerman et al, *Biotechniques*, 21: 268-279 (1996); Diviacco et al, *Gene*, 122: 3013-3020 (1992); Becker-Andre et al, *Nucleic Acids Research*, 17: 9437-9446 (1989); and the like.

[0034] “Polynucleotide” or “oligonucleotide” is used interchangeably and each means a linear polymer of nucleotide monomers. Monomers making up polynucleotides and oligonucleotides are capable of specifically binding to a natural polynucleotide by way of a regular pattern of monomer-to-monomer interactions, such as Watson-Crick type of base pairing, base stacking, Hoogsteen or reverse Hoogsteen types of base pairing, wobble base pairing, or the like. As described in detail below, by “wobble base” is meant a nucleic acid base that can base pair with a first nucleotide base in a complementary nucleic acid strand but that, when employed as a template strand for nucleic acid synthesis, leads to the incorporation of a second, different nucleotide base into the synthesizing strand. Such monomers and their internucleosidic linkages may be naturally occurring or may be analogs thereof, e.g. naturally occurring or non-naturally occurring analogs. Non-naturally occurring analogs may include peptide nucleic acids (PNAs, e.g., as described in U.S. Patent 5,539,082, incorporated herein by reference), locked nucleic acids (LNAs, e.g., as described in U.S. Patent 6,670,461, incorporated herein by reference), phosphorothioate internucleosidic linkages, bases containing linking groups permitting the attachment of labels, such as fluorophores, or haptens, and the like. Whenever the use of an oligonucleotide or polynucleotide requires enzymatic processing, such as extension by a polymerase, ligation by a ligase, or the like, one of ordinary skill would understand that oligonucleotides or polynucleotides in those instances would not contain certain analogs of internucleosidic linkages, sugar moieties, or bases at any or some positions. Polynucleotides typically range in size from a few monomeric units, e.g. 5-40, when they are usually referred to as “oligonucleotides,” to several thousand monomeric units. Whenever a polynucleotide or oligonucleotide is represented by a sequence of letters (upper or lower case), such as “ATGCCTG,” it will be understood that the nucleotides are in 5'→3' order

from left to right and that "A" denotes deoxyadenosine, "C" denotes deoxycytidine, "G" denotes deoxyguanosine, and "T" denotes thymidine, "I" denotes deoxyinosine, "U" denotes uridine, unless otherwise indicated or obvious from context. Unless otherwise noted the terminology and atom numbering conventions will follow those disclosed in Strachan and Read, *Human Molecular Genetics 2* (Wiley-Liss, New York, 1999). Usually polynucleotides comprise the four natural nucleosides (e.g. deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine for DNA or their ribose counterparts for RNA) linked by phosphodiester linkages; however, they may also comprise non-natural nucleotide analogs, e.g. including modified bases, sugars, or internucleosidic linkages. It is clear to those skilled in the art that where an enzyme has specific oligonucleotide or polynucleotide substrate requirements for activity, e.g. single stranded DNA, RNA/DNA duplex, or the like, then selection of appropriate composition for the oligonucleotide or polynucleotide substrates is well within the knowledge of one of ordinary skill, especially with guidance from treatises, such as Sambrook et al, *Molecular Cloning, Second Edition* (Cold Spring Harbor Laboratory, New York, 1989), and like references.

[0035] "Primer" means an oligonucleotide, either natural or synthetic, that is capable, upon forming a duplex with a polynucleotide template, of acting as a point of initiation of nucleic acid synthesis and being extended from its 3' end along the template so that an extended duplex is formed. The sequence of nucleotides added during the extension process is determined by the sequence of the template polynucleotide. Usually primers are extended by a DNA polymerase. Primers are generally of a length compatible with their use in synthesis of primer extension products, and are usually are in the range of between 8 to 100 nucleotides in length, such as 10 to 75, 15 to 60, 15 to 40, 18 to 30, 20 to 40, 21 to 50, 22 to 45, 25 to 40, and so on, more typically in the range of between 18-40, 20-35, 21-30 nucleotides long, and any length between the stated ranges. Typical primers can be in the range of between 10-50 nucleotides long, such as 15-45, 18-40, 20-30, 21-25 and so on, and any length between the stated ranges. In some embodiments, the primers are usually not more than about 10, 12, 15, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, or 70 nucleotides in length.

[0036] Primers are usually single-stranded for maximum efficiency in amplification, but may alternatively be double-stranded. If double-stranded, the primer is usually first treated to separate its strands before being used to prepare extension products. This denaturation step is typically affected by heat, but may alternatively be carried out using alkali, followed by

neutralization. Thus, a “primer” is complementary to a template, and complexes by hydrogen bonding or hybridization with the template to give a primer/template complex for initiation of synthesis by a polymerase, which is extended by the addition of covalently bonded bases linked at its 3' end complementary to the template in the process of DNA synthesis.

[0037] A “primer pair” as used herein refers to first and second primers having nucleic acid sequence suitable for nucleic acid-based amplification of a target nucleic acid. Such primer pairs generally include a first primer having a sequence that is the same or similar to that of a first portion of a target nucleic acid, and a second primer having a sequence that is complementary to a second portion of a target nucleic acid to provide for amplification of the target nucleic acid or a fragment thereof. Reference to “first” and “second” primers herein is arbitrary, unless specifically indicated otherwise. For example, the first primer can be designed as a “forward primer” (which initiates nucleic acid synthesis from a 5' end of the target nucleic acid) or as a “reverse primer” (which initiates nucleic acid synthesis from a 5' end of the extension product produced from synthesis initiated from the forward primer). Likewise, the second primer can be designed as a forward primer or a reverse primer.

[0038] “Primer site” (e.g., a sequencing primer site, and amplification primer site, etc.) as used herein refers to a domain in a polynucleotide that includes the sequence of a primer (e.g., a sequencing primer) and/or the complementary sequence of a primer. When present in single stranded form (e.g., in a single stranded polynucleotide), a primer site can be either the identical sequence of a primer or the complementary sequence of a primer. When present in double stranded form, a primer site contains the sequence of a primer hybridized to the complementary sequence of the primer. Thus, a primer site is a region of a polynucleotide that is either identical to or complementary to the sequence of a primer (when in a single stranded form) or a double stranded region formed between a primer sequence and its complement. Primer sites may be present in an adapter attached to a polynucleotide. The specific orientation of a primer site can be inferred by those of ordinary skill in the art from the structural features of the relevant polynucleotide and/or context in which it is used.

[0039] “Readout” means a parameter, or parameters, which are measured and/or detected that can be converted to a number or value. In some contexts, readout may refer to an actual numerical representation of such collected or recorded data. For example, a readout of fluorescent intensity signals from a microarray is the address and fluorescence intensity of a signal being generated at each hybridization site of the microarray; thus, such a readout may

be registered or stored in various ways, for example, as an image of the microarray, as a table of numbers, or the like.

[0040] “Reflex site”, “reflex sequence” and equivalents are used to indicate sequences in a polynucleotide that are employed to move a domain intramolecularly from its initial location to a different location in the polynucleotide. The sequence of a reflex site can be added to a polynucleotide of interest (e.g., present in an adapter ligated to the polynucleotide), be based on a sequence naturally present within the polynucleotide of interest (e.g., a genomic sequence in the polynucleotide), or a combination of both. The reflex sequence is chosen so as to be distinct from other sequences in the polynucleotide (i.e., with little sequence homology to other sequences likely to be present in the polynucleotide, e.g., genomic or sub-genomic sequences to be processed). As such, a reflex sequence should be selected so as to not hybridize to any sequence except its complement under the conditions employed in the reflex processes herein described. As described later in this application, the complement to the reflex sequence is inserted on the same strand of the polynucleotide (e.g., the same strand of a double-stranded polynucleotide or on the same single stranded polynucleotide) in a particular location so as to facilitate an intramolecular binding event on such particular strand. Reflex sequences employed in the reflex process described herein can thus have a wide range of lengths and sequences. Reflex sequences may range from 5 to 200 nucleotide bases in length.

[0041] “Solid support”, “support”, and “solid phase support” are used interchangeably and refer to a material or group of materials having a rigid or semi-rigid surface or surfaces. In many embodiments, at least one surface of the solid support will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different compounds with, for example, wells, raised regions, pins, etched trenches, or the like. According to other embodiments, the solid support(s) will take the form of beads, resins, gels, microspheres, or other geometric configurations. Microarrays usually comprise at least one planar solid phase support, such as a glass microscope slide.

[0042] “Specific” or “specificity” in reference to the binding of one molecule to another molecule, such as a labeled target sequence for a probe, means the recognition, contact, and formation of a stable complex between the two molecules, together with substantially less recognition, contact, or complex formation of that molecule with other molecules. In one aspect, “specific” in reference to the binding of a first molecule to a second molecule means that to the extent the first molecule recognizes and forms a complex with another molecule

in a reaction or sample, it forms the largest number of the complexes with the second molecule. Preferably, this largest number is at least fifty percent. Generally, molecules involved in a specific binding event have areas on their surfaces or in cavities giving rise to specific recognition between the molecules binding to each other. Examples of specific binding include antibody-antigen interactions, enzyme-substrate interactions, formation of duplexes or triplexes among polynucleotides and/or oligonucleotides, biotin-avidin or biotin-streptavidin interactions, receptor-ligand interactions, and the like. As used herein, “contact” in reference to specificity or specific binding means two molecules are close enough that weak noncovalent chemical interactions, such as Van der Waal forces, hydrogen bonding, base-stacking interactions, ionic and hydrophobic interactions, and the like, dominate the interaction of the molecules.

[0043] As used herein, the term “ T_m ” is used in reference to the “melting temperature.” The melting temperature is the temperature (e.g., as measured in °C) at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. Several equations for calculating the T_m of nucleic acids are known in the art (see e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization (1985). Other references (e.g., Allawi, H.T. & SantaLucia, J., Jr., Biochemistry 36, 10581-94 (1997)) include alternative methods of computation which take structural and environmental, as well as sequence characteristics into account for the calculation of T_m .

[0044] “Sample” means a quantity of material from a biological, environmental, medical, or patient source in which detection, measurement, or labeling of target nucleic acids is sought. On the one hand it is meant to include a specimen or culture (e.g., microbiological cultures). On the other hand, it is meant to include both biological and environmental samples. A sample may include a specimen of synthetic origin. Biological samples may be animal, including human, fluid, solid (e.g., stool) or tissue, as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Biological samples may include materials taken from a patient including, but not limited to cultures, blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum, semen, needle aspirates, and the like. Biological samples may be obtained from all of the various families of domestic animals, as well as feral or wild animals, including, but not limited to, such animals as ungulates, bear, fish, rodents, etc. Environmental samples include environmental material such as surface matter, soil, water and industrial samples, as well as samples obtained from food and dairy processing

instruments, apparatus, equipment, utensils, disposable and non-disposable items. These examples are not to be construed as limiting the sample types applicable to the present invention.

[0045] The terms "upstream" and "downstream" in describing nucleic acid molecule orientation and/or polymerization are used herein as understood by one of skill in the art. As such, "downstream" generally means proceeding in the 5' to 3' direction, i.e., the direction in which a nucleotide polymerase normally extends a sequence, and "upstream" generally means the converse. For example, a first primer that hybridizes "upstream" of a second primer on the same target nucleic acid molecule is located on the 5' side of the second primer (and thus nucleic acid polymerization from the first primer proceeds towards the second primer).

[0046] It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely", "only" and the like in connection with the recitation of claim elements, or the use of a "negative" limitation.

DETAILED DESCRIPTION OF THE INVENTION

[0047] The invention is drawn to compositions and methods for intramolecular nucleic acid rearrangement that find use in various applications of genetic analysis, including sequencing, as well as general molecular biological manipulations of polynucleotide structures.

[0048] Before the present invention is described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0049] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also

encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0050] Unless defined otherwise, all technical and scientific terms used 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, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. It is understood that the present disclosure supersedes any disclosure of an incorporated publication to the extent there is a contradiction.

[0051] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a nucleic acid" includes a plurality of such nucleic acids and reference to "the compound" includes reference to one or more compounds and equivalents thereof known to those skilled in the art, and so forth.

[0052] The practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such conventional techniques include polymer array synthesis, hybridization, ligation, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the example herein below. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV), *Using Antibodies: A Laboratory Manual*, *Cells: A Laboratory Manual*, *PCR Primer: A Laboratory Manual*, and *Molecular Cloning: A Laboratory Manual* (all from Cold Spring Harbor Laboratory Press), Stryer, L. (1995) *Biochemistry* (4th Ed.) Freeman, New York, Gait, "Oligonucleotide Synthesis: A Practical Approach" 1984, IRL Press, London, Nelson and Cox (2000), Lehninger, A., *Principles of Biochemistry* 3rd Ed., W. H. Freeman Pub., New York, N.Y. and Berg et al. (2002) *Biochemistry*, 5th Ed., W. H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

[0053] 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. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

[0054] As summarized above, aspects of the present invention are drawn to the use of a 'reflex' sequence present in a polynucleotide (e.g., in an adapter structure of the polynucleotide, in a genomic region of the polynucleotide, or a combination of both) to move a domain of the polynucleotide intra-molecularly from a first location to a second location. The reflex process described herein finds use in any number of applications, e.g., placing functional elements of a polynucleotide (e.g., sequencing primer sites and/or MID tags) into proximity to a desired sub-region of interest.

Nucleic Acids

[0055] The reflex process (as described in detail below) can be employed for the manipulation and analysis of nucleic acid sequences of interest from virtually any nucleic acid source, including but not limited to genomic DNA, complementary DNA (cDNA), RNA (e.g., messenger RNA, ribosomal RNA, short interfering RNA, microRNA, etc.), plasmid DNA, mitochondrial DNA, synthetic DNA, etc. Furthermore, any organism, organic material or nucleic acid-containing substance can be used as a source of nucleic acids to be processed in accordance with the present invention including, but not limited to, plants, animals (e.g., reptiles, mammals, insects, worms, fish, etc.), tissue samples, bacteria, fungi (e.g., yeast), phage, viruses, cadaveric tissue, archaeological/ancient samples, etc. In certain embodiments, the nucleic acids in the nucleic acid sample are derived from a mammal, where in certain embodiments the mammal is a human.

[0056] In certain embodiments, the nucleic acid sequences are enriched prior to the reflex sequence process. By enriched is meant that the nucleic acid is subjected to a process that reduces the complexity of the nucleic acids, generally by increasing the relative concentration of particular nucleic acid species in the sample (e.g., having a specific locus of interest, including a specific nucleic acid sequence, lacking a locus or sequence, being within a specific size range, etc.). There are a wide variety of ways to enrich nucleic acids having a specific characteristic(s) or sequence, and as such any convenient method to accomplish this may be employed. The enrichment (or complexity reduction) can take place at any of a number of steps in the process, and will be determined by the desires of the user. For

example, enrichment can take place in individual parental samples (e.g., untagged nucleic acids prior to adaptor ligation) or in multiplexed samples (e.g., nucleic acids tagged with primer sites, MID and/or reflex sequences and pooled; MID are described in further detail below).

[0057] In certain embodiments, nucleic acids in the nucleic acid sample are amplified prior to analysis. In certain of these embodiments, the amplification reaction also serves to enrich a starting nucleic acid sample for a sequence or locus of interest. For example, a starting nucleic acid sample can be subjected to a polymerase chain reaction (PCR) that amplifies one or more region of interest. In certain embodiments, the amplification reaction is an exponential amplification reaction, whereas in certain other embodiments, the amplification reaction is a linear amplification reaction. Any convenient method for performing amplification reactions on a starting nucleic acid sample can be used in practicing the subject invention. In certain embodiments, the nucleic acid polymerase employed in the amplification reaction is a polymerase that has proofreading capability (e.g., phi29 DNA Polymerase, Thermococcus litoralis DNA polymerase, Pyrococcus furiosus DNA polymerase, etc.).

[0058] In certain embodiments, the nucleic acid sample being analyzed is derived from a single source (e.g., a single organism, virus, tissue, cell, subject, etc.), whereas in other embodiments, the nucleic acid sample is a pool of nucleic acids extracted from a plurality of sources (e.g., a pool of nucleic acids from a plurality of organisms, tissues, cells, subjects, etc.), where by "plurality" is meant two or more. As such, in certain embodiments, a nucleic acid sample can contain nucleic acids from 2 or more sources, 3 or more sources, 5 or more sources, 10 or more sources, 50 or more sources, 100 or more sources, 500 or more sources, 1000 or more sources, 5000 or more sources, up to and including about 10,000 or more sources.

[0059] In certain embodiments, nucleic acid fragments that are to be pooled with nucleic acid fragments derived from a plurality of sources (e.g., a plurality of organisms, tissues, cells, subjects, etc.), where by "plurality" is meant two or more. In such embodiments, the nucleic acids derived from each source includes a multiplex identifier (MID) such that the source from which the each tagged nucleic acid fragment was derived can be determined. In such embodiments, each nucleic acid sample source is correlated with a unique MID, where by unique MID is meant that each different MID employed can be differentiated from every other MID employed by virtue of at least one characteristic, e.g., the nucleic acid sequence

of the MID. Any type of MID can be used, including but not limited to those described in co-pending U.S. Patent Application Serial Number 11/656,746, filed on January 22, 2007, and titled "Nucleic Acid Analysis Using Sequence Tokens", as well as U.S. Patent 7,393,665, issued on July 1, 2008, and titled "Methods and Compositions for Tagging and Identifying Polynucleotides", both of which are incorporated herein by reference in their entirety for their description of nucleic acid tags and their use in identifying polynucleotides. In certain embodiments, a set of MIDs employed to tag a plurality of samples need not have any particular common property (e.g., T_m , length, base composition, etc.), as the asymmetric tagging methods (and many tag readout methods, including but not limited to sequencing of the tag or measuring the length of the tag) can accommodate a wide variety of unique MID sets.

[0060] In certain embodiments, each individual polynucleotide (e.g., double-stranded or single-stranded, as appropriate to the methodological details employed) in a sample to be analyzed is tagged with a unique MID so that the fate of each polynucleotide can be tracked in subsequent processes (where, as noted above, unique MID is meant to indicate that each different MID employed can be differentiated from every other MID employed by virtue of at least one characteristic, e.g., the nucleic acid sequence of the MID). For example (and as described below), having each nucleic acid tagged with a unique MID allows analysis of the sequence of each individual nucleic acid using the reflex sequence methods described herein. This allows the linkage of sequence information for large nucleic acid fragments that cannot be sequenced in a single sequencing run.

Reflex Sequence Process

[0061] As summarized above, aspects of the present invention include methods and compositions for moving a domain in a polynucleotide from a first location to a second location in the polynucleotide. An exemplary embodiment is shown in Figure 1A.

[0062] Figure 1A shows a single stranded polynucleotide 100 comprising, in a 5' to 3' orientation, a first domain (102; the domain to be moved); a reflex sequence 104; a nucleic acid sequence 106 having a site distal to the first domain (Site A), and a complement of the reflex sequence 108 (positioned at the 3' terminus of the polynucleotide). The steps of the reflex method described below will move the first domain into closer proximity to Site A. It is noted here that the prime designation in Figure 1A denotes a complementary sequence of a domain. For example, First Domain' is the complement of the First Domain.

[0063] In Step 1, the reflex sequence and its complement in the polynucleotide are annealed intramolecularly to form polynucleotide structure 112, with the polynucleotide folding back on itself and hybridizing to form a region of complementarity (i.e., double stranded reflex/reflex' region). In this configuration, the 3' end of the complement of the reflex sequence can serve as a nucleic acid synthesis priming site. Nucleic acid synthesis from this site is then performed in extension Step 2 producing a complement of the first domain at the 3' end of the nucleic acid extension (shown in polynucleotide 114; extension is indicated with dotted arrow labeled "extend").

[0064] Denaturation of polynucleotide 114 (e.g., by heat) generates linear single stranded polynucleotide 116. As shown in Figure 1, resultant polynucleotide 116 contains a complement of the first domain at a position proximal to Site A (i.e., separated by only the complement of the reflex sequence). This resultant polynucleotide may be used for any subsequent analysis or processing steps as desired by the user (e.g., sequencing, as a template for amplification (linear, PCR, etc.), sequence specific extraction, etc.).

[0065] In alternative embodiments, the first domain and reflex sequence are removed from the 5' end of the double-stranded region of polynucleotide 114 (shown in polynucleotide 118; removal is shown in the dotted arrow labeled "remove"). Removal of this region may be accomplished by any convenient method, including, but not limited to, treatment (under appropriate incubation conditions) of polynucleotide structure 114 with T7 exonuclease or by treatment with Lambda exonuclease; the Lambda exonuclease can be employed so long as the 5' end of the polynucleotide is phosphorylated. If the region is removed enzymatically, resultant polynucleotide 118 is used in place of polynucleotide 116 in subsequent steps (e.g., copying to reverse polarity).

[0066] In certain embodiments, polynucleotide 116 or 118 is used as a template to produce a double stranded polynucleotide, for example by performing a nucleic acid synthesis reaction with a primer that primes in the complement of the first domain. This step is sometimes referred to as copying to reverse polarity of a single stranded polynucleotide, and in some instances, the double-stranded intermediate product of this copying is not shown (see, e.g., Figure 3). For example, copying to reverse the polarity of polynucleotide 116 results in single-stranded polynucleotide 120 having, in a 5' to 3' orientation, the first domain (122); the reflex sequence (124); the complement of polynucleotide 106 (oriented with the complement of Site A (Site A'; 126) proximal to the reflex sequence); the complement of the reflex sequence (128); and the complement of the first domain (130).

[0067] In certain embodiments, the first domain in the polynucleotide comprises one or more elements that find use in one or more subsequent processing or analysis steps. Such sequences include, but are not limited to, restriction enzyme sites, PCR primer sites, linear amplification primer sites, reverse transcription primer sites, RNA polymerase promoter sites (such as for T7, T3 or SP6 RNA polymerase), MID tags, sequencing primer sites, etc. Any convenient element can be included in the first domain and, in certain embodiments, is determined by the desires of the user of the methods described herein.

[0068] As an exemplary embodiment, suppose we want to sequence a specific polynucleotide region from multiple genomes in a pooled sample where the polynucleotide region is too long to sequence in a single reaction. For example, sequencing a polynucleotide region that is 2 kilobases or more in length using Roche 454 (Branford, CT) technology, in which the length of a single sequencing run is about 400 bases. In this scenario, we can design a set of left hand primers (A_n) and right hand primers (B_n) specific for the polynucleotide region that are positioned in such a way that we can obtain direct sequences of all parts of the insert, as shown in Figure 1B. Note that the polynucleotide shown in Figure 1B (140) has a domain (142) containing a primer site and an MID denoting from which original sample(s) the polynucleotide is derived. Site 142 thus represents an example of a First Domain site such identified as 122 in the Figure 1A. The polynucleotide also includes a reflex site (144), which can be part of the polynucleotide region itself (e.g., a genomic sequence), added in a ligated adapter domain along with the primer site and the MID (an artificial sequence), or a combination of both (a sequence spanning the adapter/polynucleotide junction).

[0069] It is noted here that polynucleotide 140 can be categorized as a precursor to polynucleotide 100 in Figure 1A, as it does not include a 3' reflex sequence complementary to the reflex site (domain 108 in Figure 1A). As detailed below, polynucleotide 140 can be converted to a polynucleotide having the structural configuration of polynucleotide 100, a polynucleotide suitable as a substrate for the reflex process described herein (e.g., by primer extension using a B_n primer and reversal of polarity).

[0070] In an exemplary embodiment, each A_n - B_n primer pair defines a nucleic acid region that is approximately 400 bases in length or less. This size range is within the single-sequencing run read length of the current Roche 454 sequencing platform; a different size range for the defined nucleic acid region may be utilized for a different sequencing platform. Thus, each product from each reflex process can be sequenced in a single run. It is noted

here that primer pairs as shown in Figure 1B can be used to define regions 1 to 5 shown in Figure 3 (described in further detail below).

[0071] In certain embodiments, to obtain the first part of the sequence of the polynucleotide region (i.e., in the original structure, that part of the polynucleotide closest to the first domain), we only need a right hand primer (e.g., B₀) and we do not need to transfer the MID as it is within reach of this sequencing primer (i.e., the MID is within 400 bases of sequencing primer B₀). All other B_n primers have the reflex sequence added to their 5' ends ("R" element shown on B primers) so that they read 5' reflex-B_n. However, in certain embodiments, the B₀ primer does include the reflex sequence and is used in the reflex process (along with a corresponding A₀ primer) as detailed below.

[0072] As described above, we obtain a single stranded polynucleotide having, in the 5' to 3' orientation, a primer site (e.g., for Roche 454 sequencing), an MID, a reflex sequence and the polynucleotide to be sequenced. Numerous methods for obtaining single-stranded polynucleotides of interest have been described and are known in the art, including in U.S. Patent 7,217,522, issued on May 15, 2007; U.S. Patent Application 11/377,462, filed on March 16, 2006; and U.S. Patent Application 12/432,080, filed on April 29, 2009; each of which is incorporated by reference herein in their entirety. For example, a single stranded product can be produced using linear amplification with a primer specific for the primer site of the template. In certain embodiments, the primer includes a binding moiety to facilitate isolation of the single stranded nucleic acid of interest, e.g., to immobilize the top strand on a binding partner of the binding moiety immobilized on a solid support. Removal of a hybridized, non-biotinylated strand by denaturation using heat or high pH (or any other convenient method) serves to isolate the biotinylated strand. Binding moieties and their corresponding binding partners are sometimes referred to herein as binding partner pairs. Any convenient binding partner pairs may be used, including but not limited to biotin/avidin (or streptavidin), antigen/antibody pairs, etc.

[0073] It is noted here that while the figures and description of the reflex process provided herein depict manipulations with regard to a single stranded polynucleotide, it is not necessarily required that the single stranded polynucleotide described or depicted in the figures be present in the sample in an isolated form (i.e., isolated from its complementary strand). In other words, double stranded polynucleotides may be used where only one strand is described/depicted, which will generally be determined by the user.

[0074] The implementation of a single strand isolation step using the methods described above or variations thereof (or any other convenient single strand isolation step) will generally be based on the desires of the user. One example of isolating single stranded polynucleotides is shown in Figure 2. In this Figure, a starting double stranded template (with 5' to 3' orientation shown as an arrow) is denatured and primed with a biotinylated synthesis primer specific for the primer site. After extension of the primer (i.e., nucleic acid synthesis), the sample is contacted with a solid support having streptavidin bound to it. The biotin moiety (i.e., the binding partner of streptavidin) on the extended strands will bind to the solid-phase streptavidin. Denaturation and washing is then performed to remove all non-biotinylated polynucleotide strands. If desired, the bound polynucleotide, which can be used in subsequent reflex process steps (e.g., as a template for B_n primer extension reactions), may be eluted from the streptavidin support. Alternatively, the bound polynucleotide may be employed in subsequent steps of the desired process while still bound to the solid support (e.g., in solid phase extension reactions using B_n primers). This process, with minor variations depending on the template being used and the identity of the desired single stranded polynucleotide, may be employed at any of a number of steps in which a single stranded product is to be isolated. It is noted that in certain embodiments, substrate bound biotinylated polynucleotide can be used to produce and isolate non-biotinylated single stranded products (i.e., by eluting the non-biotinylated products while leaving the biotinylated templates bound to the streptavidin on the solid support). Thus, the specifics of how binding partners are used to isolate single stranded polynucleotides of interest will vary depending on experimental design parameters.

Additional single-stranded isolation/production methods include asymmetric PCR, strand-specific enzymatic degradation, and the use of in-vitro transcription followed by reverse transcriptase (IVT-RT) with subsequent destruction of the RNA strand. As noted above, any convenient single stranded production/isolation method may be employed.

[0075] To the single stranded polynucleotide shown in Figure 1B we anneal one of the B_n primers having the appended reflex sequence, denoted with a capital "R" (e.g., B₁) and extend the primer under nucleic acid synthesis conditions to produce a copy of the polynucleotide that has a reflex sequence at its 5' end. A single stranded copy of this polynucleotide is then produced to reverse polarity using a primer specific for the primer site in the first domain' (complement of the first domain 102). The resulting nucleic acid has structure 100 shown in Figure 1A, where the first domain 102 includes the primer site and

the MID. Site A (110) in Figure 1 is determined by the specificity of the 5' reflex-B_n primer used.

[0076] The reflex process (e.g., as shown in Figure 1) is then performed to produce a product in which the primer site and the MID are now in close proximity to the desired site (or region of interest (ROI)) within the original polynucleotide (i.e., the site defined by the primer used, e.g., B₁). The resulting polynucleotide can be used in subsequent analyses as desired by the user (e.g., Roche 454 sequencing technology).

[0077] It is noted here that, while not shown in Figure 1A and 1B, any convenient method for adding adapters to a polynucleotide to be processed as described herein may be used in the practice of the reflex process (adapters containing, e.g., primer sites, polymerase sites, MIDs, restriction enzyme sites, and reflex sequences). For example, adapters can be added at a particular position by ligation. For double stranded polynucleotides, an adapter can be configured to be ligated to a particular restriction enzyme cut site. Where a single stranded polynucleotide is employed, a double stranded adapter construct that possesses an overhang configured to bind to the end of the single-stranded polynucleotide can be used. For example, in the latter case, the end of a single stranded polynucleotide can be modified to include specific nucleotide bases that are complementary to the overhang in the double stranded adaptor using terminal transferase and specific nucleotides. In other embodiments, PCR or linear amplification methods using adapter-conjugated primers is employed to add an adapter at a site of interest. Again, any convenient method for producing a starting polynucleotide may be employed in practicing the methods of the subject invention.

[0078] In certain embodiments, the nucleic acid may be sequenced directly using a sequencing primer specific for the primer site. This sequencing reaction will read through the MID and desired site in the insert.

[0079] In certain embodiments, the polynucleotide may be isolated (or fractionated) using an appropriate A_n primer (e.g., when using B₁ as the first primer, primer A₁ can be used). In certain embodiments, the A_n primed polynucleotide is subjected to nucleic acid synthesis conditions to produce a copy of the fragment produced in the reflex process. In certain of these embodiments, the A_n primer has appended on its 5' end a primer site that can be used in subsequent steps, including sequencing reactions. Providing a primer site in the A_n primer allows amplifying and/or sequencing from both ends of the resultant fragment: from the primer site in the first domain 102 and the primer site in the A_n primer (not shown in Figure 1B). Because of the position of the primer sites and their distance apart (i.e., less than one

sequencing run apart), sequencing from both ends will usually capture the sequence of the desired site (or ROI) and the sequence of the MID, which can be used for subsequent bioinformatic analyses, e.g., to positively identify the sample of origin. It is noted here that while sequencing in both directions is possible, it is not necessary, as sequencing from either primer site alone will capture the sequence of the ROI as well as its corresponding MID sequence.

[0080] Note that in certain embodiments, the first fragment obtained by amplification/extension from primer B₀ directly, the polarity of the ROI in the resulting fragment is reversed as compared to the ROI in fragments obtained by primers B₁-B_n. This is because the B₀-generated fragment, unlike the B₁-B_n generated fragments, has not been subjected to a reflex process which reverses the orientation of the ROI sequence with respect to the first domain/reflex sequence (as described above). Therefore, the B₀ primer may have appended to it a primer site (e.g., at its 5' end) that can be used for subsequent amplification and/or sequencing reactions (e.g., in Roche 454 sequencing system) rather than a reflex sequence as with primers B₁-B_n. However, in certain embodiments, as noted above, the reflex process may be used with a corresponding B₀-A₀ primer pair as described above, i.e., using a B₀ primer having a 5' reflex sequence and a corresponding A₀ primer with its corresponding 5' adapter domain (e.g., a primer site).

[0081] It is noted here that because the particular sections of sequence to be analyzed are defined by the A_n-B_n primer pairs (as shown and described above), a much higher sequence specificity is achieved as compared to using previous extraction methods that employ only a single oligo binding event (e.g., using probes on a microarray).

[0082] Figure 3 provides a detailed flow chart for an exemplary embodiment that employs reflex sequences for use in sequencing multiple specific regions in a polynucleotide (i.e., regions 1, 2, 3, 4 and 5 in an 11kb region of lambda DNA).

[0083] A single parent DNA fragment 202 is generated that includes adapter domains (i.e., a Roche 454 sequencing primer site, a single MID, and a reflex sequence) and the sequence of interest. In the example shown, the sequence of interest is from lambda DNA and the reflex sequence is present on the top strand (with its complement shown in the bottom strand). Any convenient method for producing this parent DNA fragment may be used, including amplification with a primer that includes the adapter domains (e.g., using PCR), cloning the fragment into a vector that includes the adapter domains (e.g., a vector with the adapter domains adjacent to a cloning site), or by attaching adapters to polynucleotide

fragments (e.g., fragment made by random fragmentation, by sequence-specific restriction enzyme digestion, or combinations thereof). While only a single fragment with a single MID is shown, the steps in Figure 3 are applicable to samples having multiple different fragments each with a different MID, e.g., a sample having a population of homologous fragments from any number of different sources (e.g., different individuals). Figure 3 describes the subsequent enzymatic steps involved in creating the five daughter fragments in which regions 1, 2, 3, 4 and 5 (shown in polynucleotide 204) are rearranged to be placed within a functional distance of the adapter domains (i.e., close enough to the adapter domains to be sequenced in a single Roche 454 sequencing reaction). Note that certain steps are shown for region 4 only (206).

[0084] In step 1, the five regions of interest are defined within the parent fragment (labeled 1 to 5 in polynucleotide 204) and corresponding primer pairs are designed for each. The distance of each region of interest from the reflex sequence is shown below polynucleotide 204. The primer pairs are designed as described and shown in Figure 1B (i.e., the A_n-B_n primer pairs). For clarity, only primer sites for region 4 are shown in Figure 3 (“primer sites” surrounding region 4). In step 2, sequence specific primer extensions are performed (only region 4 is shown) with corresponding B_n primers to produce single stranded polynucleotides having structure 208 (i.e., having the reflex sequence on the 5' terminus). As shown, the B_n primer for region 4 will include a sequence specific primer site that primes at the 3'-most primer site noted for region 4 (where “3'-most” refers to the template strand, which in Figure 3 is the top strand). This polynucleotide is copied back to produce polynucleotide 210 having reversed polarity (e.g., copied using a primer that hybridizes to the 454A' domain). Polynucleotide 210 has structure similar to polynucleotide 100 shown at the top of Figure 1. Step 4 depicts the result of the intramolecular priming between the reflex sequence and its complement followed by extension to produce the MID' and 454A' structures at the 3' end (polynucleotide 212). In the embodiments shown in Figure 3, polynucleotide 212 is treated with T7 exonuclease to remove double stranded DNA from the 5' end (as indicated above, this step is optional). The polynucleotide formed for region 4 is shown as 216 with polynucleotides for the other regions also shown (214).

[0085] It is noted here that the formation of each of the polynucleotides 214 may be accomplished either in separate reactions (i.e., structure with region 1 in proximity to the adapter domains is in a first sample, the structure with region 2 in proximity to the adapter region is in a second sample, etc.) or in one or more combined sample.

[0086] In step 6 the polynucleotides 214 are copied to reverse polarity to form polynucleotides 218. In step 7, each of these products are then primed with the second primer of the specific primer pair (see A_n primers as shown in Figure 1B) each having a second Roche 454 primer site (454B) attached at the 5' end, and extended to form products 220. Steps 6 and 7 may be combined (e.g., in a single PCR or other amplification reaction).

[0087] In summary, Figure 3 shows how the reflex process can be employed to produce five daughter fragments 220 of similar length (e.g., ~500 bp) each of which contain DNA sequences that differ in their distance from the reflex sequence in the starting structure 202 while maintaining the original MID.

[0088] Figure 4 shows another exemplary use of the reflex process as described herein. In the embodiment shown in Figure 4, a target sequence (i.e., containing region of interest "E") is enriched from a pool of adapter-attached fragments. In certain embodiments, the fragments are randomly sheared, selected for a certain size range (e.g., DNA having a length from 100 to 5000 base pairs), and tagged with adapters (e.g., asymmetric adapters, e.g., as described in US Patent Application Ser. No. 12/432,080, filed on April 29, 2009). The asymmetric adaptor employed in Figure 4 contains a sequencing primer site (454A, as used in the Roche 454 sequencing platform), an MID, an X sequence, and an internal stem region (ISR), which denotes the region of complementarity for the asymmetric adapter that is adjacent to the adapter attachment site (see, e.g., the description in US Application Ser. No. 12/432,080, filed on April 29, 2009, incorporated herein by reference in its entirety). The X sequence can be any sequence that can serve as a binding site for a polynucleotide containing the complement of the X sequence (similar to a primer site). As described below, the X sequence allows for the annealing of an oligonucleotide having a 5' overhang that can serve as a template for extension of the 3' end of the adaptor oligonucleotide. The sequencing direction of the sequencing primer site (454A primer site in structure 401 of Figure 4) is oriented such that amplification of the adapter ligated fragment using the sequencing primer site proceeds away from the ligated genomic insert. This has the effect of making the initial asymmetric adapter ligated library 'inert' to amplification using this primer, e.g., in a PCR reaction.

[0089] To extract a region of interest (the "E" region), the library is mixed with an oligonucleotide (403) containing a 3' X' sequence and a target specific priming sequence (the 1' sequence) under hybridization/annealing conditions. The target specific sequence 1' is designed to flank one side of the region of interest (the 1' sequence adjacent to E in the

genomic insert; note that only the E-containing polynucleotide fragment is shown in Figure 4), much like a PCR primer. After annealing primer 403, the hybridized complex is extended, whereby all of the adaptor tagged fragments will obtain the complement of the target specific sequence (i.e., the 1 sequence) on the 3' end (see structure 405; arrows denote the direction of extension).

[0090] Extended products 405 are then denatured and the 1/1' regions allowed to hybridize intramolecularly in a reflex process priming event, after which nucleic acid extension is performed to form structure 407 (extension is from the 1 priming site; shown with an arrow). This reflex reaction creates a product (407) that, unlike its parent structure (405), has a sequencing primer site (454A) that is oriented such the extension using this primer sequence proceeds towards the region of interest. Thus, in the absence of a priming and extension reflex reaction, extension with a sequencing primer will not generate a product containing the region of interest (the E region). In other words, only E-region containing target polynucleotides will have a 454A sequence that can amplify genomic material (structure 407).

[0091] After completing the reflex process (using 1/1' as the reflex sequences), a PCR amplification reaction is performed to amplify the region of interest (with associated adapter domains). However, before performing the PCR reaction, the fragment sample is "inactivated" from further extension using terminal transferase and ddNTPs. This inactivation prevents non-target adaptor tagged molecules from performing primer extension from the 3' primer 1 site. Once inactivated, a PCR reaction is performed using a sequencing primer (i.e., 454A primer 409) and a second primer that primes and extends from the opposite side of the region of interest (i.e., primer 411, which includes a 5' 454B sequencing primer site and a 3' "2" region that primes on the opposite end of E from the 1 region). Only fragments that have undergone the reflex process and contain the E region will be suitable templates for the PCR reaction and produce the desired product (413).

[0092] Thus, the process exemplified in Figure 4 allows for the movement of an adapter domain (e.g., containing functional elements and/or MID) into proximity to a desired region of interest.

[0093] The reflex process described herein can be used to perform powerful linkage analysis by combining it with nucleic acid counting methods. Any convenient method for tagging and /or counting individual nucleic acid molecules with unique tags may be employed (see, e.g., U.S. Patent 7,537,897, issued on May 26, 2009; U.S. Patent 7,217,522,

issued on May 15, 2007; U.S. Patent Application 11/377,462, filed on March 16, 2006; and U.S. Patent Application 12/432,080, filed on April 29, 2009; each of which is incorporated by reference herein in their). All of this can be conducted in parallel thus saving on the cost of labor, time and materials.

[0094] In one exemplary embodiment, a large collection of sequences is tagged with MID such that each polynucleotide molecule in the sample has a unique MID. In other words, each polynucleotide in the sample (e.g., each individual double stranded or single stranded polynucleotide) is tagged with a MID that is different from every other MID on every other polynucleotide in the sample. In general, to accomplish such molecular tagging the number of distinct MID tags to be used should be many times greater than the actual number of molecules to be analyzed. This will result in the majority of individual nucleic acid molecules being labeled with a unique ID tag (see, e.g., Brenner et al., Proc. Natl. Acad. Sci. USA. 2000 97(4):1665-70). Any sequences that then result from the reflex process on that particular molecule (e.g., as described above) will thus be labeled with the same unique MID tag and thus inherently linked. Note that once all molecules in a sample are individually tagged, they can be manipulated and amplified as much as needed for processing so long as the MID tag is maintained in the products generated.

[0095] For example, we might want to sequence one thousand viral genomes (or a specific genomic region) or one thousand copies of a gene present in somatic cells. After tagging each polynucleotide in the sample with a sequencing primer site, MID and reflex sequence (as shown in the figures and described above), we use the reflex process to break each polynucleotide into lengths appropriate to the sequencing procedure being used, transferring the sequencing primer site and MID to each fragment (as described above). Obtaining sequence information from all of the reflex-processed samples can be used to determine the sequence of each individual polynucleotide in the starting sample, using the MID sequence to defining linkage relationships between sequences from different regions in the polynucleotide being sequenced. Using a sequencing platform with longer read lengths can minimize the number of primers to be used (and reflex fragments generated).

[0096] The advantages noted above are shown in Figure 5. This figure shows a comparison of methods for identifying nucleic acid polymorphisms in homologous nucleic acids in a sample (e.g., the same region derived from a chromosomal pair of a diploid cell or viral genomes/transcripts). The top schematic shows two nucleic acid molecules in a sample (1 and 2) having a different assortment of polymorphisms in polymorphic sites A, B and C

(A1, B1, C1 and C2). Standard sequencing methods using fragmentation (left side) can identify the polymorphisms in these nucleic acids but do not retain linkage information. Employing the reflex process described herein to identify polymorphisms (right side) maintains linkage information. It is noted that not all domain structures and steps are shown in the reflex process for simplicity.

Kits and Systems

[0097] Also provided by the subject invention are kits and systems for practicing the subject methods, as described above, such vectors configured to add reflex sequences to nucleic acid inserts of interest and reagents for performing any steps in the cloning or reflex process described herein (e.g., restriction enzymes, nucleotides, polymerases, primers, exonucleases, etc.). The various components of the kits may be present in separate containers or certain compatible components may be precombined into a single container, as desired.

[0098] The subject systems and kits may also include one or more other reagents for preparing or processing a nucleic acid sample according to the subject methods. The reagents may include one or more matrices, solvents, sample preparation reagents, buffers, desalting reagents, enzymatic reagents, denaturing reagents, where calibration standards such as positive and negative controls may be provided as well. As such, the kits may include one or more containers such as vials or bottles, with each container containing a separate component for carrying out a sample processing or preparing step and/or for carrying out one or more steps of a nucleic acid variant isolation assay according to the present invention.

[0099] In addition to above-mentioned components, the subject kits typically further include instructions for using the components of the kit to practice the subject methods, e.g., to prepare nucleic acid samples for perform the reflex process according to aspects of the subject methods. The instructions for practicing the subject methods are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or sub-packaging) etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the

instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

[0100] In addition to the subject database, programming and instructions, the kits may also include one or more control samples and reagents, e.g., two or more control samples for use in testing the kit.

Utility

[0101] The reflex process described herein provides significant advantages in numerous applications, a few of which are noted below (as well as described above).

[0102] For example, as described above, certain aspects of the reflex process define the particular sections of sequence to be analyzed by a primer pair, as in PCR (e.g., the two oligos shown as A_n-B_n in Figure 1B). This results in higher sequence specificity as compared to other extraction methods (e.g., using probes on a microarray) that only use a single oligo sequence. The separation of the probes defines a length that can be relatively uniform (hence making subsequent handling including amplification more uniform) and can also be tailored to the particular sequencing platform being employed.

[0103] Further, as described above, aspects of the present invention can be used to analyze homologous genomic locations in a multiplexed sample (i.e., a sample having polynucleotides from different genomic samples) in which the polynucleotides are tagged with the MID. This is possible because the reflex process, which operates intramolecularly, maintains the MID thus linking any particular fragment to the sample from which it originates.

[0104] Finally, as the reflex processes described herein function intramolecularly, one can determine the genetic linkage between different regions on the same large fragment that are too far apart to be sequenced in one sequence read. Such a determination of linkage may be of great value in plant or animal genetics (e.g., to decide if a particular set of variations are linked together on the same stretch of chromosome) or in viral studies (e.g., to determine if particular variations are linked together on the same stretch of a viral genome/transcripts, e.g., HIV, hepatitis virus, etc.).

EXAMPLES

Example I

[0105] Figures 6 and 7 provide experimental data and validation of the reflex process described herein using synthetic polynucleotide substrates.

METHODS

Substrate:

[0106] The 100 base oligonucleotide substrate (as shown diagrammatically in Figure 6A) was synthesized with internal fluorescein-dT positioned between the REFLEX and REFLEX' sequences. This label provides convenient and sensitive method of detection of oligonucleotide species using polyacrylamide gel electrophoresis.

Extension reactions:

[0107] Reactions were prepared which contained 1 μ M of the 100 base oligonucleotide substrate, 200 μ M dNTPs, presence or absence of 1 μ M competitor oligonucleotide, 0.5 μ l of each DNA polymerase ("DNAP"): Vent (NEB, 2 units/ μ l), Taq (Qiagen HotStarTaq 5 units/ μ l) and Herculanase (Stratagene), and made up to 50 μ l with the appropriate commercial buffers for each polymerase and dH₂O. For Taq titrations 0.5 μ l, 1 μ l, 2 μ l, and 3 μ l enzyme was used in the same 50 μ l volume. Reactions were heated in a Biometra thermocycler to 95 °C for 15 minutes (Taq) or 5 minutes (Herculanase, Vent), followed by 55 °C or 50 °C for 30 seconds, and a final incubation at 72 °C for 10 minutes.

T7 exonuclease digestions:

[0108] Reactions were prepared with 10 μ l extension reactions above, 0.5 μ l T7 exonuclease (NEB, 10 units/ μ l), and made up to 50 μ l using NEB Buffer 4 and dH₂O. Reactions were incubated at 25 °C for 30 minutes.

Gel Electrophoresis analysis:

[0109] An 8% denaturing polyacrylamide gel was used to analyze reaction species. 0.4 μ l of extension reactions, and 2 μ l of digestion reactions were loaded and ran at 800V for ~1.5 hours. Gels were analyzed for fluorescein using an Amersham/General Electric Typhoon imager.

RESULTS

[0110] Figure 6A shows the structure of each stage of reflex sequence processing with the expected nucleic acid size shown on the left. The initial single stranded nucleic acid having a sequencing primer site (the Roche 454 sequencing primer A site; listed as 454A); an MID; a reflex sequence; the insert; and a complement of the reflex sequence is 100 nucleotides in length. After self-annealing and extension, the product is expected to be 130 nucleotides in length. After removal of the double stranded region from the 5' end, the nucleic acid is expected to be 82 bases in length.

[0111] Figure 6B shows the results of three experiments using three different nucleic acid polymerases (Vent, Herculanase and Taq, indicated at the top of the lanes). The temperature at which the annealing was carried out is shown at the top of each lane (either 50°C or 55°C). The sizes of the three nucleic acids as noted above are indicated on the left and right side of the gel.

[0112] As shown in figure 6B, extension appears to be most efficient under the conditions used with Herculanase (Herculanase is a mixture of two enzymes: modified Pfu DNAP and Archaeumax (dUTPase)). Most (or all) of the initial 100 base pair nucleic acid are converted to the 130 base pair product (see lanes 6 and 7). However, after T7 exonuclease digestion the 3'-5' exonuclease activity of Herculanase results in partial digestion of the desired 82 base product (note bands at and below the 82 base pairs in lanes 8 and 9).

[0113] Taq, which lacks 3'-5' exonuclease activity, shows a stronger band at the expected size of the final product after T7 exonuclease digestion (see lane 13).

[0114] Figure 7 shows the effect on the reflex process of increasing amounts of Taq polymerase as well as the use of a reflex sequence competitor (schematically shown in Figure 7A).

[0115] As shown in lanes 2 to 5, increased Taq concentration improves extension to ~90% conversion of the starting nucleic acid (see lane 5). Lanes 7 to 8 show that T7 exonuclease digestion does not leave a perfect 82 base product. This may be due to collapse of dsDNA when T7 exonuclease has nearly completed its digestion from the 5' end in the double stranded region of the fold-back structure. It is noted that in many embodiments, the removal of a few additional bases from the 5' end of the polynucleotide will not interfere with subsequent analyses, as nucleotide bases at the 5' end are often removed during subsequent steps.

[0116] As shown in Lanes 11-14, addition of a competitor (which can interfere with annealing of the reflex sequences to form the fold-back structure) results in only a small decrease (~5-10%) of fully extended product. Thus, as expected, the intramolecular reaction is heavily favored. Although not shown, we have observed that the competitor oligonucleotide also gets extended by the same amount (~5-10%).

[0117] The concentration of the competitor, the concentration of the reflex substrate, and the overall genetic complexity, will all likely affect specific results. The experiments shown in Figures 6 and 7 demonstrate that the core parts of the reflex processes as described herein is functional and can be implemented.

Example II

[0118] Figure 8 shows the reflex workflow (diagram at left) and exemplary results of the workflow (gel at right) for a specific region of interest (ROI). The starting material is a double stranded nucleic acid molecule (700) that contains a 454A primer site, an MID, a reflex site, and a polynucleotide of interest having three ROIs (2, 3 and 4) at different locations therein. This starting material was subjected to reflex processes (as described in above) specific for ROI 2 as shown in the diagram at the left of the figure, both with and without the use of a T7 exonuclease step (the T7 exonuclease step is shown in the diagram is indicated as “Optional”).

[0119] Completion of all steps shown in the reflex process should result in a double stranded polynucleotide of 488 base pairs (702) with or without the T7 exonuclease step.

[0120] As shown in the gel on the right of Figure 8, the 488 base pair product was produced in reflex processes with and without the T7 exonuclease step.

[0121] Figure 9 shows an exemplary protocol for a reflex process based on the results discussed above. The diagram shows specific reflex process steps with indications on the right as to where purification of reaction products is employed (e.g., using Agencourt SPRI beads to remove primer oligos). One reason for performing such purification steps is to reduce the potential for generating side products in a reaction (e.g., undesirable amplicons). While Figure 9 indicates three purification steps, fewer or additional purification steps may be employed depending on the desires of the user. It is noted that the steps of reversing polarity, reflex priming and extension, and “stretch out” (or denaturation) / second reversing polarity step can be performed without intervening purification steps.

[0122] The protocol shown in Figure 9 includes the following steps:

annealing a first primer containing a 5' reflex sequence (or reflex tail, as noted in the figure) specific for the 3' primer site for the R' region to the starting polynucleotide and extending (the primer anneals to the top strand at the primer site at the right of R in polynucleotide 902, indicated with a *; this step represents the first denature, anneal and extend process indicated on the right);

after purification, adding a 454A primer and performing three cycles of denaturing, annealing and extending: the first cycle results in the copy-back from the 454A primer to reverse the polarity of the strand just synthesized; the second cycle breaks apart the double stranded structure produced, allows the reflex structure to form and then extend; the third cycle results in another copy-back using the same 454A primer originally added;

after purification, adding a second primer specific for the second primer site for the R' region having a 5' 454B tail (this primer anneals to the primer site 3' of the R' region in polynucleotide 904, indicated with a *) and denaturing, annealing and extending resulting in a polynucleotide product having 454A and 454B sites surrounding the MID, the reflex sequence, and R'. Note that the first primer specific for the R' region and the second primer specific for the R' region define its boundaries, as described above and depicted in Figure 1B);

after another purification, adding 454A and 454B primers and performing a PCR amplification reaction.

Example III

[0123] As described above, a reflex sequence can be an “artificial” sequence added to a polynucleotide as part of an adapter or can be based on a sequence present in the polynucleotide of interest being analyzed, e.g., a genomic sequence (or “non-artificial”).

[0124] The data shown in prior Examples used “artificial” reflex sites. In this Example, the reflex site is a genomic sequence present in the polynucleotide being analyzed.

[0125] The starting material is a double stranded DNA containing a 454A site, an MID and a polynucleotide to be analyzed. The 454A and MID were added by adapter ligation to parent polynucleotide fragments followed by enrichment of the polynucleotide to be analyzed by a hybridization-based pull-out reaction and subsequent secondary PCR amplification (see Route 1 in Figure 13). Thus, the reflex site employed in this example is a sequence normally present at the 5' end of the subject polynucleotide (a genomic sequence). The polynucleotide being analyzed includes a region of interest distal to the 454A and MID sequences that is 354 base pairs in length.

[0126] This starting double stranded nucleic acid is 755 base pairs in length. Based on the length of each of the relevant domains in this starting nucleic acid, the reflex process should result in a product of 461 base pairs.

[0127] Figure 10 shows the starting material for the reflex process (left panel) and the resultant product generated using the reflex process (right panel; reflex process was performed as described in Example II, without using a T7 exonuclease step). A size ladder is included in the left hand lane of each gel to allow estimation of the size of the test material. This figure shows that the 755 base pair starting nucleic acid was processed to the expected 461 base pair product, thus confirming that a “non-artificial” reflex site is effective

in moving an adapter domain from one location to another in a polynucleotide of interest in a sequence specific manner.

Example IV

[0128] Figure 11 shows a schematic of an experiment in which the reflex process is performed on a single large initial template (a “parent” fragment) to generate 5 different products (“daughter” products) each having a different region of interest (i.e., daughter products are produced having either region 1, 2, 3, 4 or 5). The schematic in Figure 11 shows the starting fragment (11,060 base pairs) and resulting products (each 488 base pairs) generated from each of the different region of interest-specific reflex reactions (reflex reactions are performed as described above). The panel (gel) on the bottom of Figure 11 shows the larger starting fragment (Lane 1) and the resulting daughter products for each region-specific reflex reaction (lanes 2 to 6, with the region of interest noted in each in the box), where the starting and daughter fragments have the expected lengths. Sequencing of the products confirmed the identity of the region of interest in each of the reflex products shown in the gel. These results demonstrate that multiple different reflex products can be generated from a single, asymmetrically tagged parent fragment while maintaining the adapter domains (e.g., the primer sites and MID).

Example V

[0129] Figure 12 details experiments performed to determine the prevalence of intramolecular rearrangement (as desired in the reflex process) vs. intermolecular rearrangement. Intermolecular rearrangement is undesirable because it can lead to the transfer of an MID from one fragment to another (also called MID switching). MID switching can occur if a reflex sequence in a first fragment hybridizes to its complement in a second fragment during the reflex process, leading to appending the MID from the second fragment to the first fragment. Thus, intermolecular rearrangement, or MID switching, should be minimized to prevent the transfer of an MID from one fragment in the sample to another, which could lead to a misrepresentation of the source of a fragment.

[0130] To measure the prevalence of MID switching under different reflex conditions, fragments having different sizes were generated that included two different MIDs, as shown in the top panel of Figure 12. The common sequence on these fragments serves as the priming site for the first extension reaction to add the second reflex sequence (see, e.g., step 2 of Figure 3). Three exemplary fragments are shown in Figure 12 for each different fragment size (i.e., 800 base pairs with an MIDB and MIDA combination; 1900 base pairs

with MIDC and MIDA combination; and 3000 base pairs with MIDD and MIDA combination). For each MID family (A, B, C and D), there are 10 different members (i.e., MIDA had 10 different members, MIDB has 10 different members, etc.). A set of 10 dual MID fragments for each different size fragment (i.e., 800, 1900 and 3000 base pairs) were generated, where the MID pairs (i.e., MIDA/MIDB, MIDA/MIDC, and MIDA/MIDD) were designated as 1/1, 2/2, 3/3, 4/4, 5/5, 6/6, 7/7, 8/8, 9/9, and 10/10. All 10 fragments of the same size were then mixed together and a reflex protocol was performed.

[0131] Due to the domain structure of the fragments, a successful reflex process results in the two MIDs for each fragment being moved to within close enough proximity to be sequenced in a single read using the Roche 454 sequencing platform (see the reflex products shown in the schematic of Figure 12). The reflex reactions for each fragment size were performed at four different fragment concentrations to determine the effect of this parameter, as well as fragment length, in the prevalence of MID switching. The reflex products from each reaction performed were subjected to 454 sequencing to determine the identity of both MIDs on each fragment, and thereby the proportion of MID switching that occurred.

[0132] The panel on the bottom left of Figure 12 shows the rate of MID switching (Y axis, shown in % incorrect (or switched) MID pair) for each different length fragment at each different concentration (X axis; 300, 30, 3 and 0.3 nM). As shown in this panel, the MID switch rate decreases with lower concentrations, as would be expected, because intermolecular, as opposed to intramolecular, binding events are concentration dependent (i.e., lower concentrations lead to reduced intermolecular hybridization/binding). In addition, the MID switch rate decreases slightly with length. This is somewhat unexpected as the ends of longer DNA fragments are effectively at a lower concentration with respect to one another. The reasons for why we do not see this is probably because the production of reflex priming intermediates continues during the final PCR, which means that reflex priming reactions are happening continuously which contributes to MID switching. It is probably the case that the shorter reflex products are able to undergo a higher rate of 'background' reflexing, and therefore increase the overall MID switch rate a little.

[0133] These results demonstrate that MID switching can be minimized (e.g., to below 2%, below 1% or even to nearly undetectable levels) by altering certain parameters of the reaction, e.g., by reducing fragment concentration and/or fragment length.

[0134] The panel on the bottom right of Figure 12 shows the frequency of MID switching in the reflex process for the 800 base pair fragments (i.e., MIDA/MIDB containing

fragments). In this figure, the area of each circle is proportional to the number of reads containing the corresponding MIDA and MIDB species (e.g., MIDA1/MIDB1; MIDA1/MIDB2; etc.). Thus, a circle representing 200 reads will be 40 times larger in terms of area than a circle representing 5 reads.

[0135] As noted above, the MIDA/MIDB combinations having the same number (shown on the X and Y axis, respectively) represent the MIDA/MIDB combinations present in the sample prior to the reflex process being performed (i.e., MIDA/MIDB combinations 1/1, 2/2, 3/3, 4/4, 5/5, 6/6, 7/7, 8/8, 9/9, and 10/10 were present in the starting sample). All other MIDA/MIDB combinations identified by Roche 454 sequencing were the result of MID switching.

[0136] This figure shows that the MID switching that occurs during the reflex process is random, i.e., that MID switching is not skewed based on the identity of the MIDs in the reaction).

Exemplary Reflex Protocols

[0137] Figure 13 shows a diagram of exemplary protocols for performing the reflex process on pools of nucleic acids, for example, pools of nucleic acids from different individuals, each of which are labeled with a unique MID. In Route 3, a pooled and tagged extended library is subjected directly to a reflex process. In Route 2, the pooled library is enriched by target-specific hybridization followed by performing the reflex process. In Route 1 employs enrichment by PCR amplification. As shown in Figure 13, PCR enrichment can be performed directly on the pooled tagged extended library or in a secondary PCR reaction after a hybridization-based enrichment step has been performed (as in Route 2) to generate an amplicon substrate that is suitable for the reflex process. Additional routes for preparing a polynucleotide sample for performing a reflex process can be implemented (e.g., having additional amplification, purification, and/or enrichment steps), which will generally be dependent on the desires of the user.

[0138] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

[0139] Accordingly, the preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which,

although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

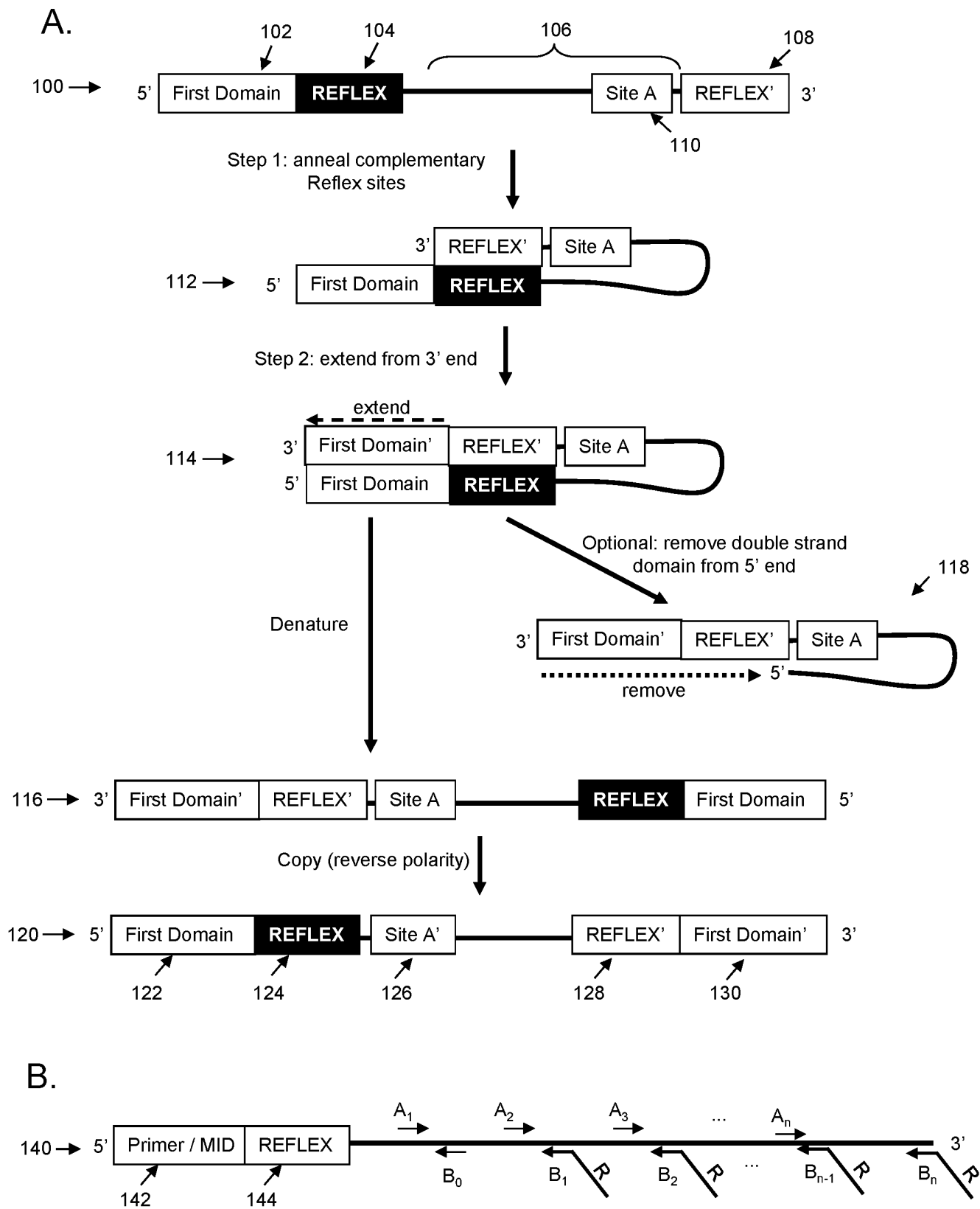


Fig. 1

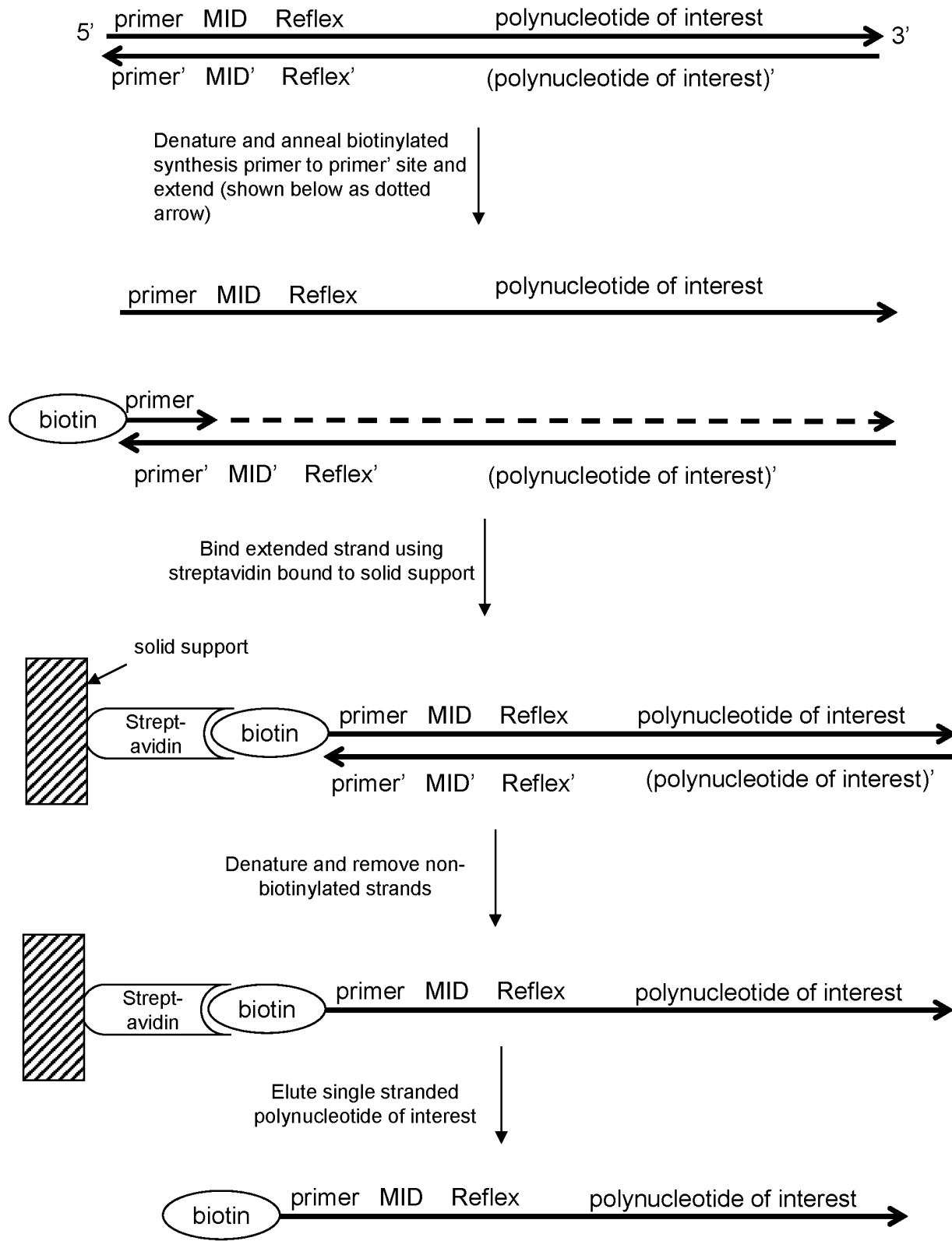
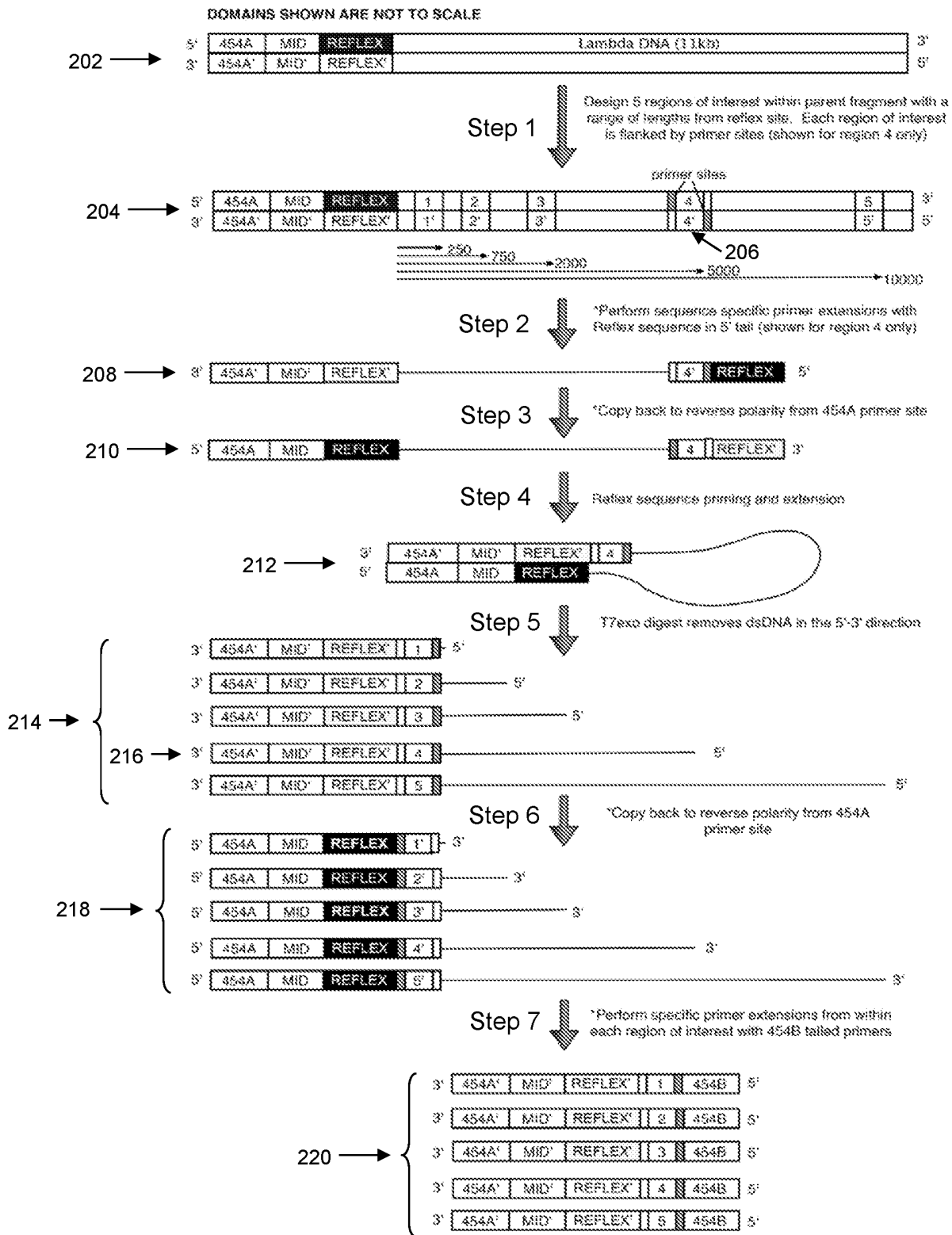


Fig. 2



Primer extension reactions with * may be performed such that isolation of single strand species is facilitated (e.g., using primers with binding moieties and/or multiple cycles of extension)

Fig. 3

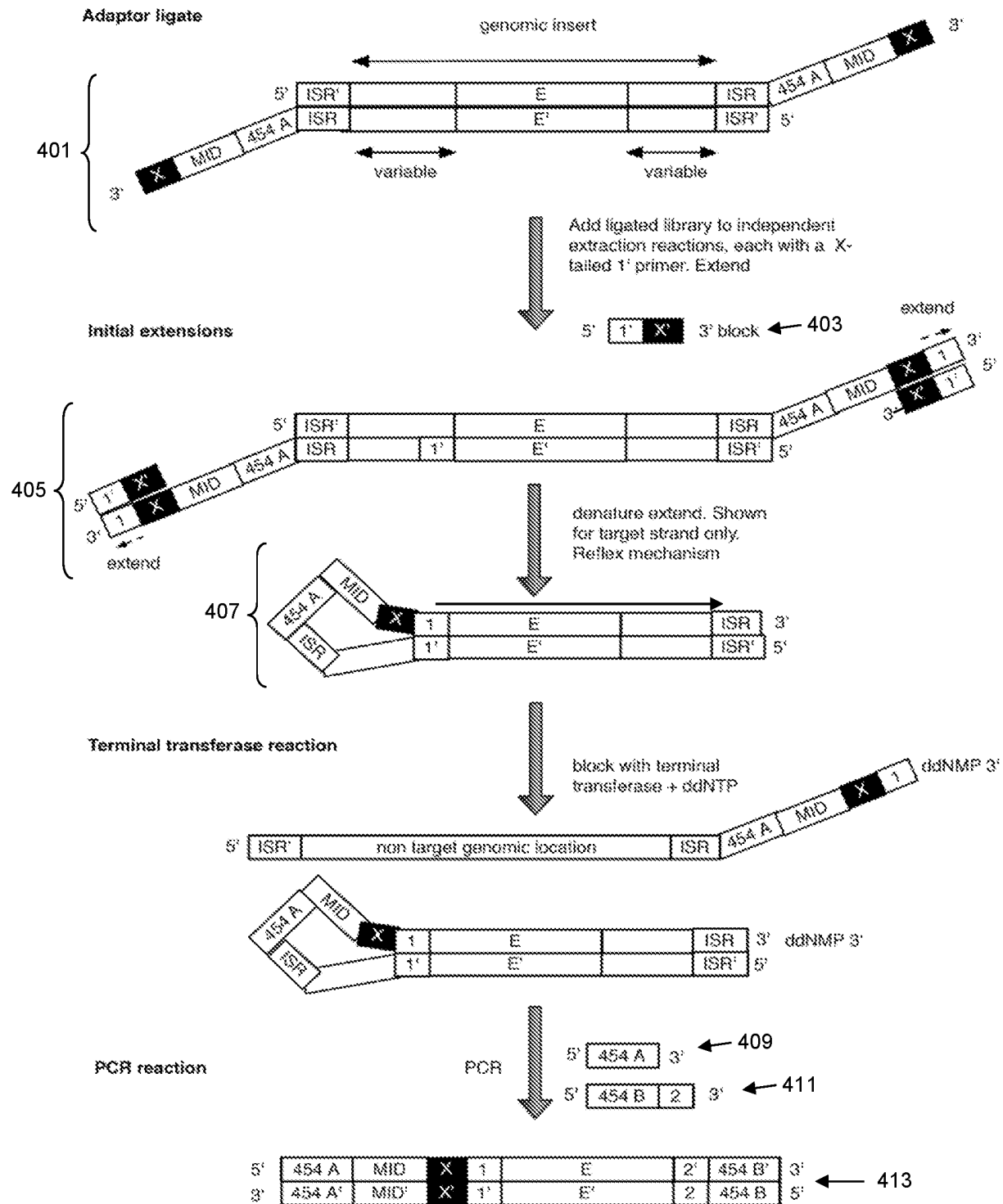


Fig. 4

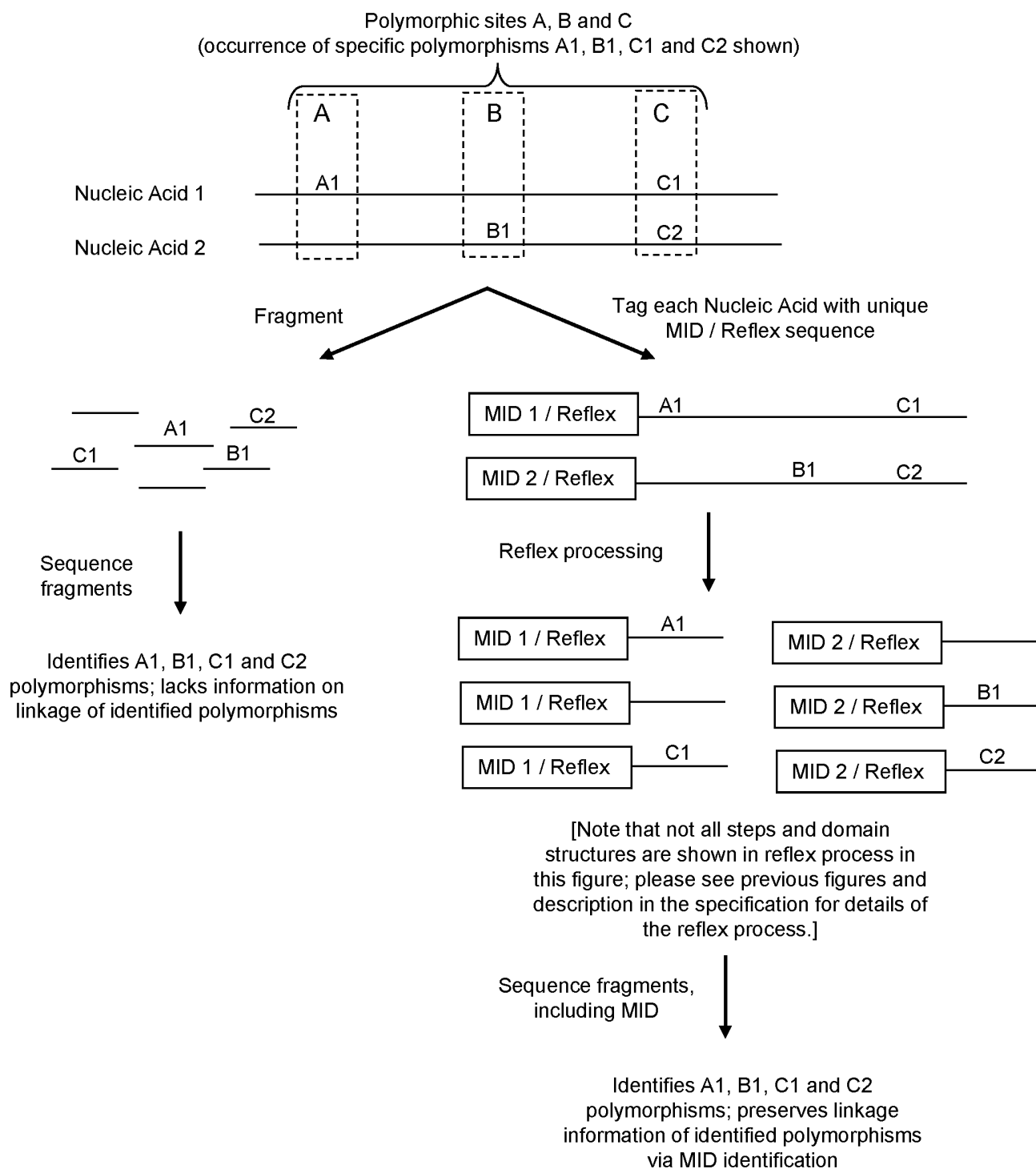
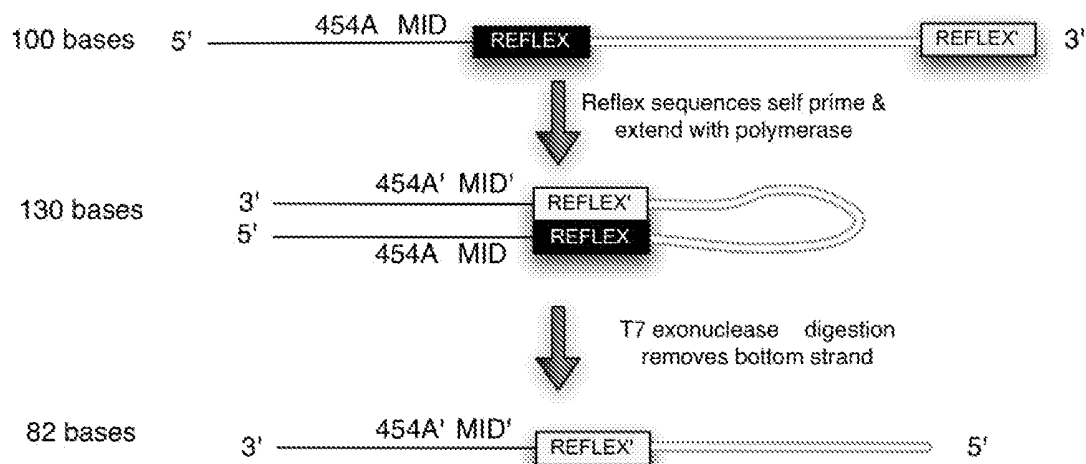


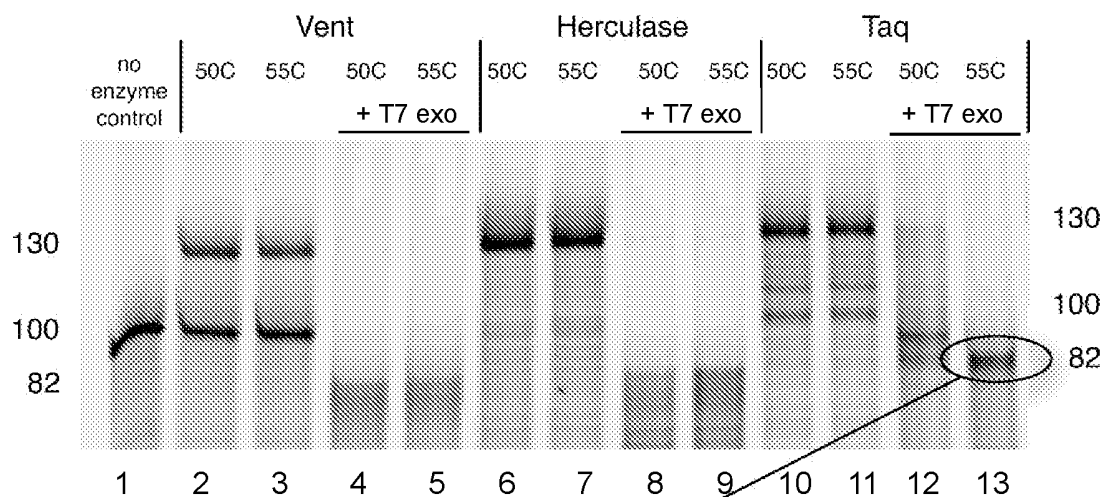
Fig. 5

A.



B.

Polymerase testing at two annealing temperatures



Extension is best with Herculase, but 3'-5' exonuclease activity results in partial digestion of the desired 82 base product. Taq, which lacks 3'-5' exonuclease activity, shows a stronger band at the expected size of the final product.

Fig. 6

A.



B.

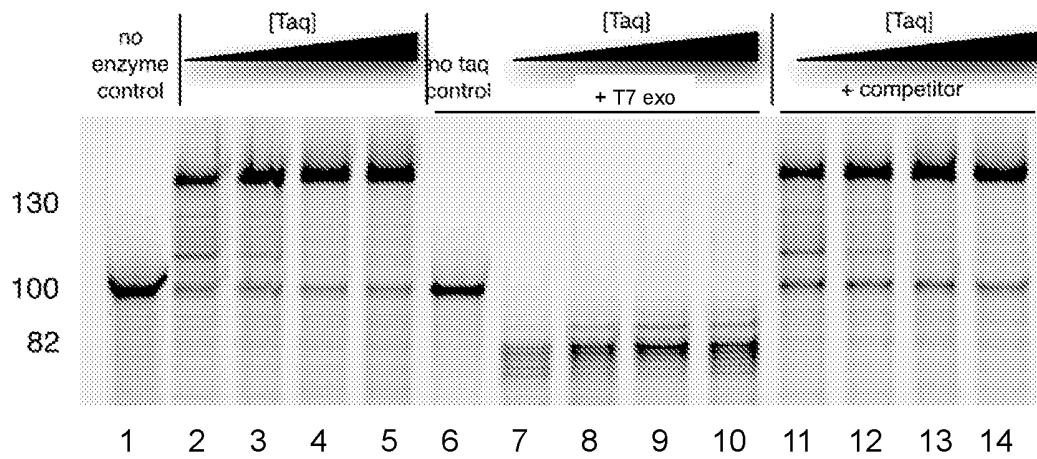
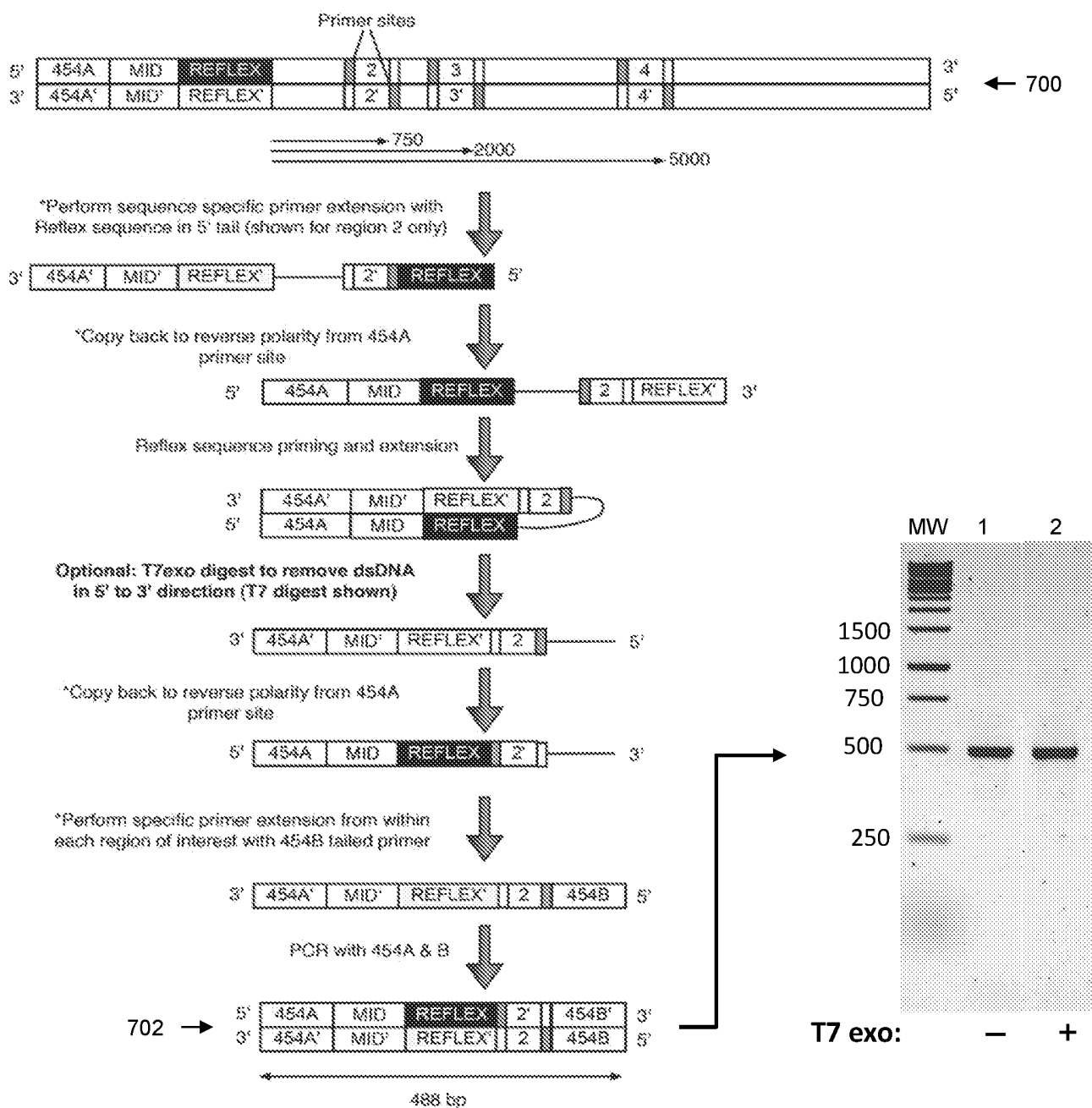


Fig. 7



Primer extension reactions with * may be performed such that isolation of single strand species is facilitated (e.g., using primers with binding moieties and/or multiple cycles of extension)

Fig. 8

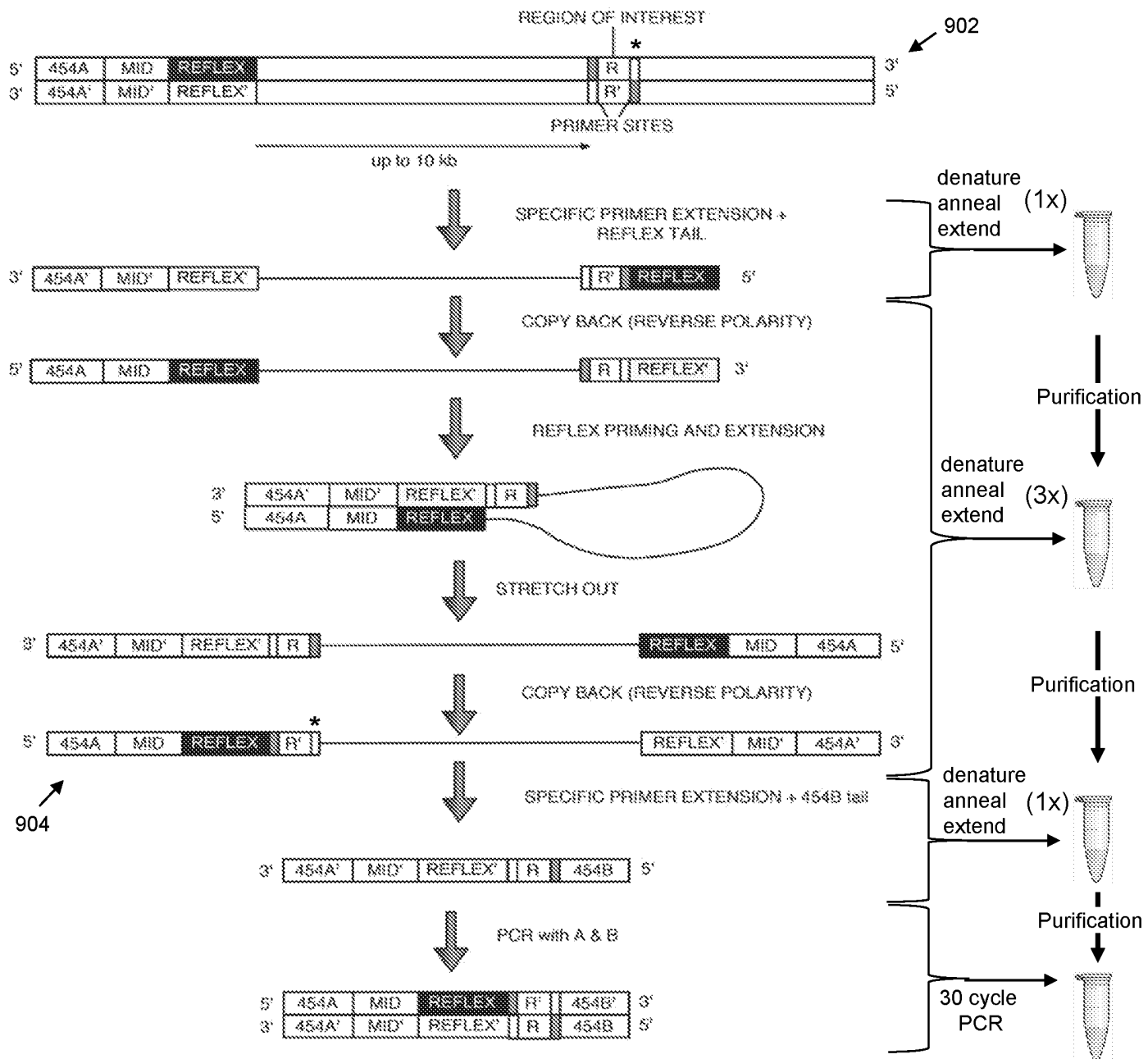


Fig. 9

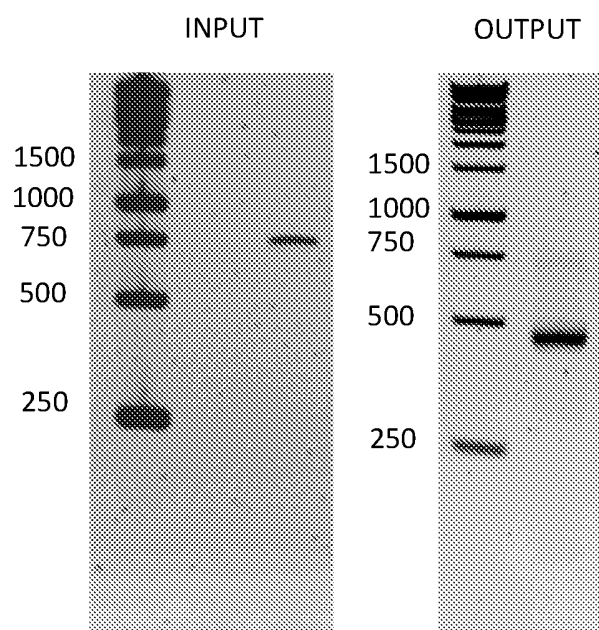


Fig. 10

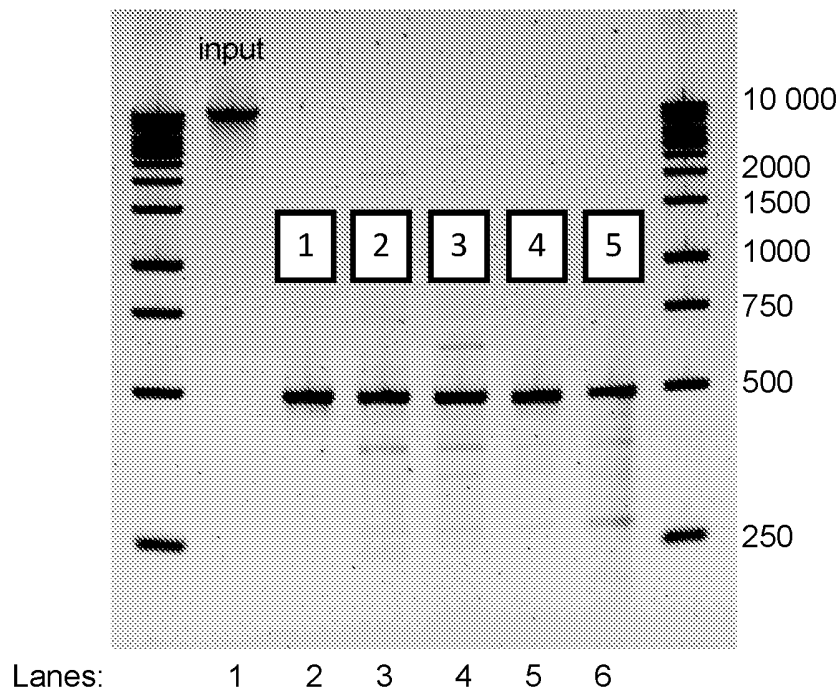
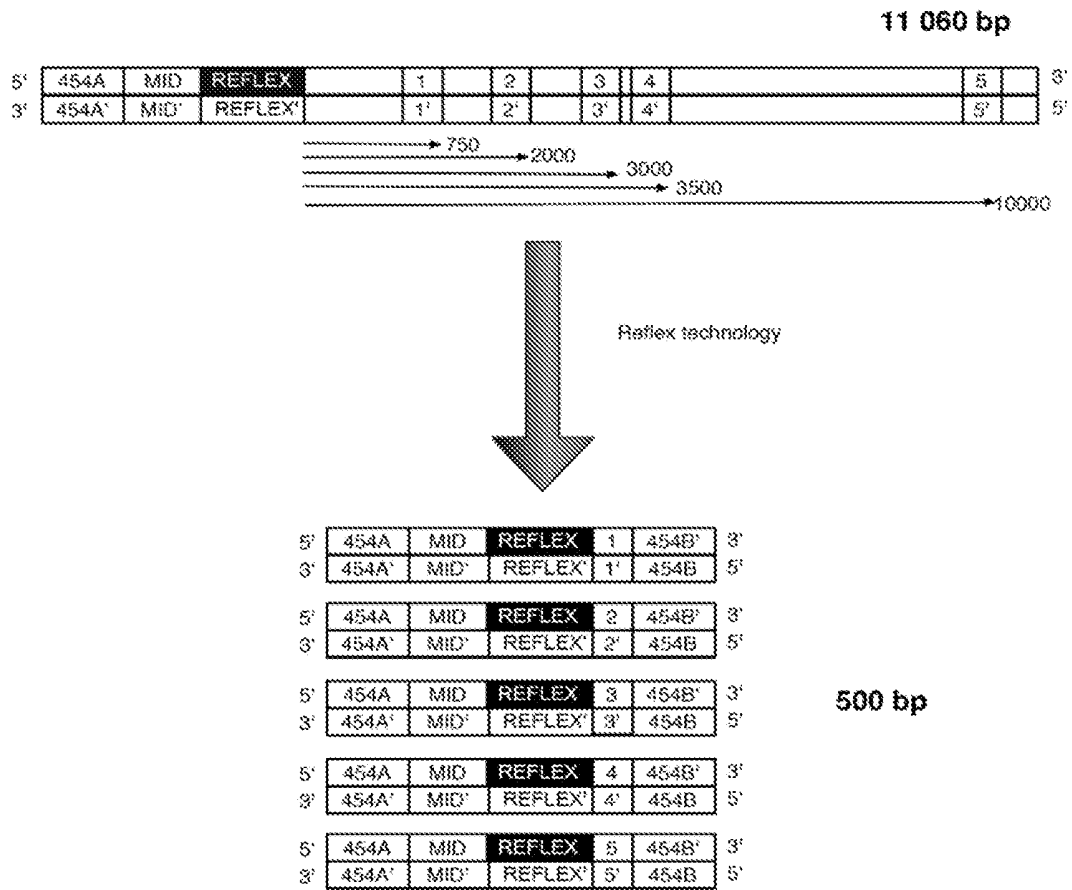
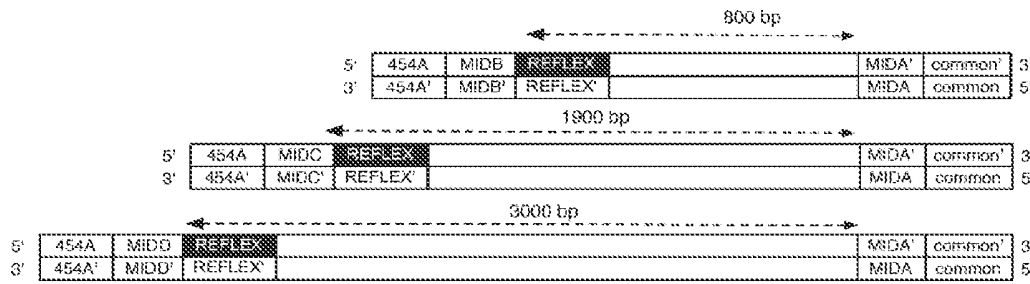
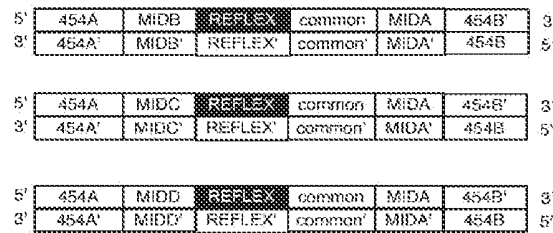


Fig. 11



For each length there are 10 pairs of MIDs

Reflex technology at four different concentrations



Roche 454 Sequencing

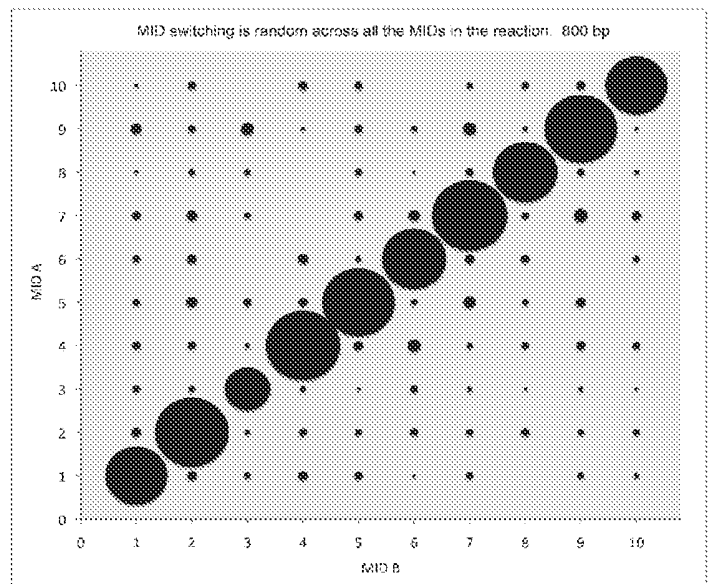
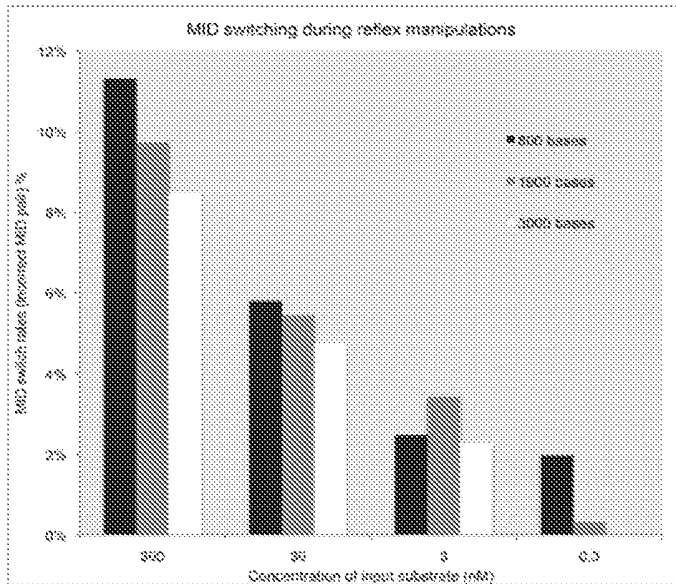


Fig. 12

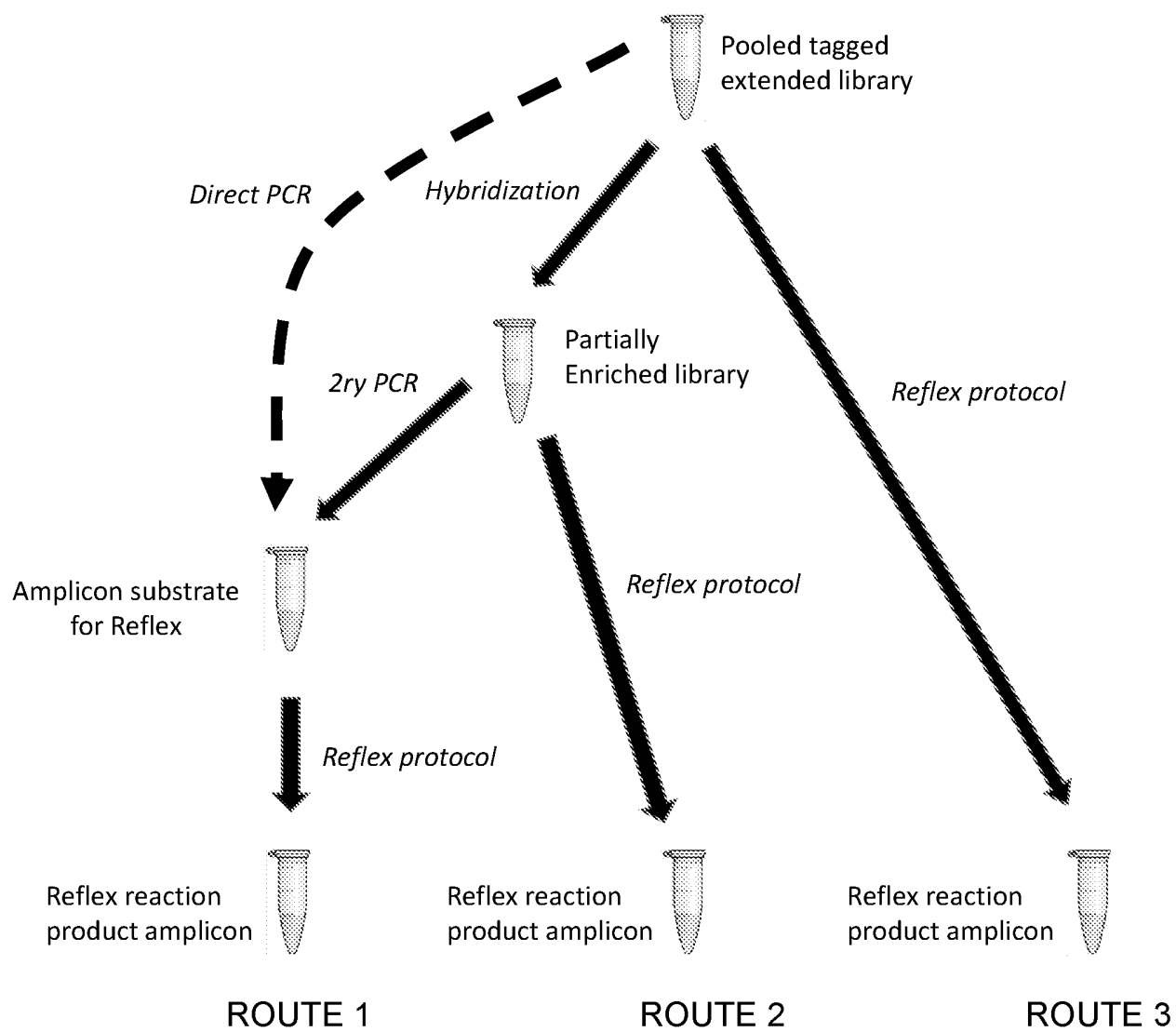


Fig. 13

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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 Intramolecular Nucleic Acid Rearrangement
---------------------------	---

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 13622872filed on September 19, 2012

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 INVENTORInventor: Sydney Brenner

Date (Optional): _____

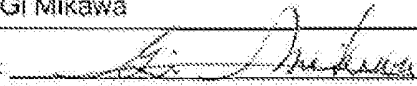
Signature: Sydney Brenner

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Application Number:				
Filing Date:				
Title of Invention:		METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		
First Named Inventor/Applicant Name:		Sydney BRENNER		
Filer:		Gargi Talukder/Leonor Rivera-Huerta		
Attorney Docket Number:		104371-5016-US10		
Filed as Small Entity				
Filing Fees for Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
UTILITY FILING FEE (ELECTRONIC FILING)	4011	1	75	75
UTILITY SEARCH FEE	2111	1	330	330
UTILITY EXAMINATION FEE	2311	1	380	380
REQUEST FOR PRIORITIZED EXAMINATION	2817	1	2000	2000
Pages:				
Claims:				
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
PROCESSING FEE, EXCEPT PROV. APPLS.	2830	1	70	70
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				2855

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International Application Number:	
Confirmation Number:	9126
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First Named Inventor/Applicant Name:	Sydney BRENNER
Customer Number:	156830
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Attorney Docket Number:	104371-5016-US10
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Application Type:	Utility under 35 USC 111(a)

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Warnings:					
Information:					
2	Transmittal of New Application	104371_5016US10_Utility_Transmittal.pdf	280356	no	2
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			f04099db213431be30f12b9e3ebdd7088b696927		
Warnings:					
Information:					
7	Fee Worksheet (SB06)	fee-info.pdf	40124	no	2
			dbff176c279ae6607c07b8f52bd82238728c78e4		
Warnings:					
Information:					
Total Files Size (in bytes):			5625686		
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.					

**CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION
UNDER 37 CFR 1.102(e) (Page 1 of 1)**

First Named Inventor:	Sydney Brenner	Nonprovisional Application Number (if known):	
Title of Invention:	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently \$0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.
2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.
3. The applicable box is checked below:

I. ☒ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

- i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.
---OR---
(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.
- ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, or the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

- i. A request for continued examination has been filed with, or prior to, this form.
- ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
- iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
- iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
- v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature <u>/Gargi Talukder/</u>	Date <u>November 16, 2018</u>
Name (Print/Typed) <u>Gargi Talukder</u>	Practitioner Registration Number <u>61,368</u>
Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.*	
☒ *Total of <u>1</u> forms are submitted.	

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 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.

SCORE Placeholder Sheet for IFW Content

Application Number: 16194047

Document Date: 11/16/2018

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via eDAN using the Supplemental Content tab, or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number
16/194,047

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	75		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	330		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	380		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	15 minus 20 = *	*	x 50 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 = *	*	x 230 =	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	785		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)	(Column 2)	(Column 3)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
		CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
AMENDMENT A	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	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
16/194,047	11/16/2018	1637	785	104371-5016-US10	15	1

CONFIRMATION NO. 9126

FILING RECEIPT



0000000104324208

156830
MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics
One Market, Spear Street Tower, Suite 2800
San Francisco, CA 94105

Date Mailed: 12/11/2018

Receipt is acknowledged of this non-provisional 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 APPLICANT, 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 Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections 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**

Inventor(s)

Sydney Brenner, Ely, UNITED KINGDOM;
Gi Mikawa, Great Shelford, UNITED KINGDOM;
Robert Osborne, Great Chesterford, UNITED KINGDOM;
Andrew Slatter, London, UNITED KINGDOM;

Applicant(s)

10X Genomics, Inc., Pleasanton, CA;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 15/677,957 08/15/2017 PAT 10155981
which is a CON of 14/792,094 07/06/2015 ABN
which is a CON of 14/172,694 02/04/2014 PAT 9102980
which is a CON of 14/021,790 09/09/2013 PAT 8679756
which is a CON of 13/859,450 04/09/2013 PAT 8563274
which is a CON of 13/622,872 09/19/2012 ABN
which is a CON of 13/387,343 02/15/2012 PAT 8298767
which is a 371 of PCT/IB2010/002243 08/13/2010
which claims benefit of 61/288,792 12/21/2009
and claims benefit of 61/235,595 08/20/2009

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: 12/07/2018

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/194,047**

Projected Publication Date: 03/21/2019

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

Preliminary Class

435

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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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/194,047	11/16/2018	Sydney Brenner	104371-5016-US10	9126
156830	7590	12/17/2018	EXAMINER	
MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics			DINES, KARLA A	
One Market, Spear Street Tower, Suite 2800				
San Francisco, CA 94105				
ART UNIT		PAPER NUMBER		
1639				
NOTIFICATION DATE		DELIVERY MODE		
12/17/2018		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):

donald.mixon@morganlewis.com
ipadmin@10xgenomics.com
sfipdocketing@morganlewis.com

<i>Decision Granting Request for Prioritized Examination (Track I)</i>	Application No. 16/194,047	Applicant(s) Brenner et al.	
	Examiner BRIAN W BROWN	Art Unit OPET	AIA (First Inventor to File) Status No
1. THE REQUEST FILED <u>16 November 2018</u> IS GRANTED . The above-identified application has met the requirements for prioritized examination A. ☒ for an original nonprovisional application (Track I). B. ☐ for an application undergoing continued examination (RCE). 2. The above-identified application will undergo prioritized examination. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs: A. filing a <u>petition for extension of time</u> to extend the time period for filing a reply; B. filing an <u>amendment to amend the application to contain more than four independent claims, more than thirty total claims</u>, or a multiple dependent claim; C. filing a <u>request for continued examination</u> ; D. filing a notice of appeal; E. filing a request for suspension of action; F. mailing of a notice of allowance; G. mailing of a final Office action; H. completion of examination as defined in 37 CFR 41.102; or I. abandonment of the application. Telephone inquiries with regard to this decision should be directed to BRIAN BROWN at (571)272-5338. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.			
/BRIAN W BROWN/ Petitions Examiner, OPET			

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Sydney Brenner et al.

Application No.: **16/194,047**

Filed: **November 16, 2018**

For: **METHODS FOR ANALYZING
NUCLEIC ACIDS FROM SINGLE
CELLS**

Confirmation No.: **9126**

Examiner: **Karla A. Dines**

Art Unit: **1639**

PRELIMINARY AMENDMENT

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

Commissioner:

Prior to examination of the above-referenced application, please enter the following amendments and remarks:

Amendments to the Claims being on page 2 of this paper.

Remarks begin on page 6 of this paper.

Electronic Patent Application Fee Transmittal				
Application Number:		16194047		
Filing Date:		16-Nov-2018		
Title of Invention:		METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		
First Named Inventor/Applicant Name:		Sydney Brenner		
Filer:		Louis-Vu T. Nguyen/Leonor Rivera-Huerta		
Attorney Docket Number:		104371-5016-US10		
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	2202	6	50	300
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				300

Electronic Acknowledgement Receipt

EFS ID:	34663959
Application Number:	16194047
International Application Number:	
Confirmation Number:	9126
Title of Invention:	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS
First Named Inventor/Applicant Name:	Sydney Brenner
Customer Number:	156830
Filer:	Louis-Vu T. Nguyen/Leonor Rivera-Huerta
Filer Authorized By:	Louis-Vu T. Nguyen
Attorney Docket Number:	104371-5016-US10
Receipt Date:	20-DEC-2018
Filing Date:	16-NOV-2018
Time Stamp:	21:19:34
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$ 300
RAM confirmation Number	122118INTEFSW00008558500310
Deposit Account	
Authorized User	

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

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		104371_5016US10_Preliminary_Amendment.pdf	111257	yes	7
			35f153ad72f08348665b664149ad84b7d5a98d7d		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Applicant Arguments/Remarks Made in an Amendment		6	7	
	Claims		2	5	
	Preliminary Amendment		1	1	
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30430	no	2
			00a3199147d1fdab1d7d253293461d2802a204fc		
Warnings:					
Information:					
Total Files Size (in bytes):			141687		

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.

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

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.

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.

REMARKS

Status of the Claims

After entry of this amendment, claims 1-5 and 16-36 will be pending and under consideration.

Claim Amendments

In the preliminary amendment, claim 1 has been amended, claims 6-15 have been cancelled and new claims 16-36 have been added. Exemplary support for the amendment to claim 1 and new claims 16-36 can be found in the specification as originally filed. As these amendments add no new matter and are fully supported by the specification as originally filed, entry of these amendments is respectfully requested.

Fees

Fees for 6 (six) claims in excess of 20 are submitted herewith.

Except for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any necessary fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17, which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-0310 (Order No.: 104371-5016-US10).

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested.

Respectfully submitted,
MORGAN, LEWIS & BOCKIUS LLP

Dated: <u>December 20, 2018</u>	By: <u>/Louis-Vu Nguyen/</u>
Customer No.: 43850	Louis-Vu Nguyen, Reg. No. 58,723, for
Morgan, Lewis & Bockius LLP	Gargi Talukder, Reg. No. 61,368
One Market, Spear Street Tower	
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AMENDMENT TO THE CLAIMS

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

1. (Currently Amended) A method of counting nucleic acids in a sample, the method comprising:
 - (a) providing a sample comprising a plurality of cells, wherein a cell of the plurality of cells comprises a plurality of sample polynucleotides;
 - (b) generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides of said cell and a plurality of oligonucleotide tags, wherein a tagged polynucleotide of the plurality of tagged polynucleotides comprises:
 - (i) a sample sequence from a sample polynucleotide of the plurality of sample polynucleotides;
 - (ii) a first tag sequence distinguishing said sample polynucleotide from sample polynucleotides from other cells; and
 - (iii) a second tag sequence distinguishing said sample polynucleotide from other sample polynucleotides from said cell;
 - (c) sequencing the tagged polynucleotide to determine the sample sequence ~~from said sample polynucleotide~~, the first tag sequence, and the second tag sequence; and
 - (d) using the first tag sequence and the second tag sequence to count a number of sample polynucleotides in said plurality of sample polynucleotides of said cell ~~having said sequence from said sample polynucleotide~~.
2. (Original) The method of claim 1, wherein the method further comprises amplifying the plurality of tagged polynucleotides prior to the sequencing step (c).
3. (Original) The method of claim 1, wherein the plurality of sample polynucleotides is selected from DNA and RNA.

4. (Original) The method of claim 1, wherein the plurality of sample polynucleotides comprises mRNA.
5. (Original) The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one ligation reaction.
- 6-15. (Cancelled)
16. (New) The method of claim 1, wherein an oligonucleotide tag of said plurality of oligonucleotide tags comprises said first tag sequence and said second tag sequence.
17. (New) The method of claim 1, wherein an oligonucleotide tag of said plurality of oligonucleotide tags comprises a sequence that is configured to hybridize to said sample polynucleotide.
18. (New) The method of claim 1, wherein the plurality of tagged polynucleotides is generated by (i) hybridizing an oligonucleotide tag of said plurality of oligonucleotide tags to said sample polynucleotide and (ii) extending said oligonucleotide tag or said sample polynucleotide or both.
19. (New) The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one linear amplification reaction.
20. (New) The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one reverse transcription reaction.
21. (New) The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one polymerase chain reaction (PCR).
22. (New) The method of claim 1, wherein substantially every sample polynucleotide of said plurality of sample polynucleotides is associated with the same first tag sequence.

23. (New) The method of claim 1, wherein at least 90 percent of said plurality of sample polynucleotides is associated with a unique second tag sequence.
24. (New) The method of claim 1, wherein at least 95 percent of said plurality of sample polynucleotides is associated with a unique second tag sequence.
25. (New) The method of claim 1, wherein at least 99 percent of said plurality of sample polynucleotides is associated with a unique second tag sequence.
26. (New) The method of claim 1, wherein substantially every sample polynucleotide of said plurality of sample polynucleotides is associated with a unique second tag sequence.
27. (New) The method of claim 1, wherein the number of different second tag sequences is larger than the number of sample polynucleotides.
28. (New) The method of claim 27, wherein the number of different second tag sequences is at least ten times the number of sample polynucleotides.
29. (New) The method of claim 28, wherein the number of different second tag sequences is at least one hundred times the number of sample polynucleotides.
30. (New) The method of claim 1, wherein said plurality of sample polynucleotides are randomly associated with said plurality of oligonucleotide tags to generate said plurality of tagged polynucleotides.
31. (New) The method of claim 1, wherein step (c) comprises hybridizing said tagged polynucleotide to a solid support.
32. (New) The method of claim 30, wherein said solid support is a bead.
33. (New) The method of claim 1, wherein step (d) comprises using said first tag sequence to distinguish (i) the number of sample polynucleotides having said sample sequence from said cell from (ii) the number of sample polynucleotides having said sample sequence from other cells.

34. (New) The method of claim 1, wherein step (d) comprises determining the number of different second tag sequences associated with said sample sequence, thereby estimating the number of sample polynucleotides having said sample sequence from said cell.
35. (New) The method of claim 1, wherein said plurality of sample polynucleotides comprises substantially all mRNA molecules of said cell.
36. (New) The method of claim 1, wherein said plurality of sample polynucleotides comprises a subset of polynucleotides of said cell having the same sequence.



UNITED STATES PATENT AND TRADEMARK OFFICE

USPTO Automated Interview Request (AIR)

Jan 3 2019

This paper requesting to schedule and/or conduct an interview is appropriate because:

This submission is requested to be accepted as an authorization for this interview to communicate via the internet. Recognizing that Internet communications are not secure, I hereby authorize the USPTO to communicate with the undersigned concerning scheduling of the interview via video conference, instant messaging, or electronic mail, and to conduct the interview in accordance with office practice including video conferencing.

Name(s) :
Louis T. Nguyen

S-signature:
/Louis Nguyen/

Registration Number:
58729

U.S. Application Number:
16194047

Confirmation Number:
9126

E-mail Address:
louis.nguyen@morganlewis.com

Phone Number:
4154421303

Proposed Time of Interview:
1-15-2019 2:00 PM ET

Alternative Proposed Time(s) of Interview:
1-15-2019 2:30 PM ET

Alternative Proposed Time(s) of Interview:
1-16-2019 2:00 PM ET

Preferred Interview Type:
Telephonic

I am the applicant or applicant's representative for this application.

Topic for Discussion:
Preliminary amendment

Electronic Acknowledgement Receipt	
EFS ID:	34846744
Application Number:	16194047
International Application Number:	
Confirmation Number:	9126
Title of Invention:	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS
First Named Inventor/Applicant Name:	Sydney Brenner
Customer Number:	156830
Filer:	Louis-Vu T. Nguyen/Sheila Gloss
Filer Authorized By:	Louis-Vu T. Nguyen
Attorney Docket Number:	104371-5016-US10
Receipt Date:	14-JAN-2019
Filing Date:	16-NOV-2018
Time Stamp:	17:46:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment		no			
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	104371-5016-US10_IDS.pdf	480136	no	8
			c0521450a1b5ebc6815cef6f75ae99fcf312137b		
Warnings:					

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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.	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:

Sidney BRENNERApplication No.: **16/194,047**Filed: **November 16, 2018**For: **Methods for Analyzing Nucleic Acids
from Single Cells**Conf. No.: **9126**Art Unit: **1639**Examiner: **Karla A. Dines**Docket No.: **104371-5016-US10***Certificate of Transmission (37 C.F.R. § 1.8)**I hereby certify that this correspondence is being transmitted to the
U.S. Patent and Trademark Office via the Office electronic filing
system (EFS-Web) to the USPTO on January 14, 2019.*/Sheila R. Gloss/*Sheila R. Gloss*Mail Stop AMENDMENT
Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450**INFORMATION DISCLOSURE STATEMENT**

Commissioner:

This Information Disclosure Statement is hereby submitted in accordance with 37 C.F.R. 1.98 and pursuant to Applicant's continuing duty under 37 C.F.R. 1.56 to bring any information which may be material to patentability of this application to the Examiner's attention.

Applicant makes no representation that a search has been conducted by the Applicant, or that there is not possibly more relevant art. 37 C.F.R. § 1.97(g). Applicant also makes no representation that the information submitted herewith is in fact material to patentability. The filing of this Information Disclosure Statement shall not be construed as an admission against interest in any manner. 37 C.F.R. § 1.97(h).

The Examiner's attention is directed to the reference(s) cited on the accompanying substitute for forms 1449A/PTO and PTO/SB/08. Except for U.S. Patents and U.S. Patent Application Publications, *see* 37 C.F.R. § 1.98(a)(2)(i), copies of the cited references are enclosed, unless otherwise noted below.

This Information Disclosure Statement is being filed within three months of the filing date of a national application other than a continued prosecution application, within three months of the date of entry of a national stage, before the mailing of a first Office action on the merits, or before the mailing of a first Office action after the filing of a request for continued examination. 37 C.F.R. § 1.97(b). No fee is required.

This Application relies on the earlier filing date of prior Application No. 15/667957, filed August 15, 2017; Application No. 14/792,094, filed July 6, 2015; Application No. 14/021,790, filed September 9, 2013; Application No. 13/859,450, filed April 9, 2013; Application No. 13/622,872, filed September 19, 2012; and Application No. 13/387,343, filed February 12, 2012, under 35 U.S.C. § 120. It is understood that the Examiner will consider information that was submitted to or cited by the Office in such prior applications. M.P.E.P. § 609.02(A)(2). Copies of documents previously submitted to or cited by the Office in the prior application are not required to be provided in this application and thus are not attached. 37 C.F.R. § 1.98(d).

The Commissioner is hereby authorized to charge any underpayment of the following fees associated with this communication, including any necessary fees for the submission of an information disclosure statement under 37 CFR § 1.17(p) or for the extension of time, or credit any overpayment to Deposit Account No. 50-0310.

Respectfully submitted,

Date: January 14, 2019
MORGAN LEWIS & BOCKIUS LLP
One Market, Spear Street Tower
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By: /Louis-Vu Nguyen/
Louis-Vu Nguyen, Reg. No. 58,729, for
Gargi Talukder, Reg. No. 61,368
Filed Under 37 C.F.R. § 1.34

Substitute for form 1449A/PTO (Substitute PTO/SB/8A) INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application Number	16/194,047
				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	1	of	5	Attorney Docket Number	104371-5016-US10

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	U.S. Patent Document Number-Kind Code ² (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A1	US-2003/0022231-A1	01-30-2003	WANGH	
	A2	US-2003/0050453-A1	03-13-2003	SORGE	
	A3	US-2003/0207300-A1	11-06-2003	MATRAY et al.	
	A4	US-2004/0224331	11-11-2004	CANTOR et al.	
	A5	US-2005/0250147-A1	11-10-2005	MACEVICZ	
	A6	US-2006/0008799	01-12-2006	CAI et al.	
	A7	US 2006/0177832 A1	08-10-2006	BRENNER	
	A8	US 2006/0177833 A1	08-10-2006	BRENNER	
	A9	US-2007/0020640	01-25-2007	MCCLOSKEY et al.	
	A10	US-2007/0072208-A1	03-29-2007	DRMANAC	
	A11	US-2007/0238113-A1	11-10-2007	KANDA et al.	
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	A15	US-2009/0155780	06-18-2009	XIAO et al.	
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	A18	US-2010/0120098	05-13-2010	GRUNENWALD et al.	
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	A20	US-2012/0208724	08-16-2012	STEEMERS	
	A21	US-2012/0316074-A1	12-13-2012	SAXONOV	
	A22	US-2013/0059310	03-07-2013	BRENNER et al.	
	A23	US-5846719-A	12-08-1998	BRENNER et al.	
	A24	US-6677121-B2	01-13-2004	LIZARDI et al.	
	A25	US-6723513	04-20-2004	LEXOW	
	A26	US-7217522-B2	05-15-2007	BRENNER	
	A27	US 7329493 B2	02-12-2008	CHOU et al.	

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				Examiner Name	Karla A. Dines
Sheet	2	of	5	Attorney Docket Number	104371-5016-US10

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	A28	US-7393665-B2	07-01-2008	BRENNER	
	A29	US-7537897-B2	05-26-2009	BRENNER et al.	
	A30	US-7544473	06-09-2009	BRENNER	
	A31	US-7700325	04-20-2010	CANTOR et al.	
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	A38	US-8679756-B1	03-25-2014	BRENNER et al.	
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	A40	US-9102980-B2	08-11-2015	BRENNER	
	A41	US-9290808-B2	03-22-2016	FODOR	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document Country Code ² Number ⁴ Kind Code ⁵ (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T ⁶
	A42	EP-1672064-A1	06-21-2006	Riken		
	A43	EP-1841879-A2	10-10-2007	Compass Genetics, LLC		
	A44	WO-2001090418-A1	11-29-2001	The Regents of the University of California		
	A45	WO-2003062462-A2	07-31-2003	Bosnes		
	A46	WO-2004061083-A2	07-22-2004	Amgen Inc.		
	A47	WO-2004065617-A2	08-05-2004	The Trustees of Boston University		
	A48	WO-2007018601-A1	02-15-2007	Rubicon Genomics, Inc.		
	A49	WO 2007147079 A2	12-21-2007	Living Microsystems, Inc. et al.		
	A50	WO-2008061193-A3	11-13-2008	Biospherex LLC et al.		

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				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	3	of	5	Attorney Docket Number	104371-5016-US10

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document Country Code ² Number ⁴ Kind Code ⁵ (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T ⁶
	A51	WO-2008150432-A1	12-11-2008	454 Life Sciences Corp		
	A52	WO 2010117620 A3	10-14-2010	Illumina, Inc.		
	A53	WO-2012148497-A2	11-01-2012	Robinson et al.		
	A54	WO-2013036929-A1	03-14-2013	The Board of Trustees of the Leland Stanford Jr University		

NON PATENT LITERATURE DOCUMENTS						
Examiner Initials*	Cite No. ¹	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, page(s), volume-issue number(s), publisher, city and/or country where published.				T ⁶
	A55	"Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry Basic MID Set" Genome Sequencer FLX System, Technnical Bulletin 004-2009, (Apr 1 2009) pp 1-7. URL:http://454.com/downloads/my454/documentation/technical-bulletins/TCB-09004 Using MultiplexIdentifierAdaptorsForTheGSFLXTitaniumSeriesChemistry-BasicMIDSet.pdf				
	A56	"Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry Extended MID Set" Genome Sequencer FLX System, Technnical Bulletin 005-2009, (Apr 1 2009) pp 1-7. URL:http://454.com/downloads/my454/documentation/technical-bulletins/TCB-09005 UsingMultiplexIdentifierAdaptorsForTheGSFLXTitaniumChemistry-ExtendedMIDSet.pdf				
	A57	AHERN, "Biochemical, Reagents Kits Offer Scientists Good Return on Investment" The Scientist (1995) 9(15):1-7				
	A58	BOULANGER, et al, "Massively parallel haplotyping on microscopic beads for the high-throughput phase analysis of single molecules", PLoS One, vol. 7:1-10, 2012.				
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	A63	HUG, H. et al. "Measurement of the number of molecules of a single mRNA species in a complex mRNA preparation" J Theor Biol. 2003 Apr 21; 221(4):615-24.				
	A64	IMBURGIO, et al, "Studies of promoter recognition and start site selection by T7 RNA polymerase using a comprehensive collection of promoter variants", Biochemistry., 39:10419-30, 2000.				

Examiner Signature		Date Considered	
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				Application Number	16/194,047
				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	4	of	5	Attorney Docket Number	104371-5016-US10

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	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, page(s), volume-issue number(s), publisher, city and/or country where published.	†6
	A65	KAPER, F. et al. "Whole-genome haplotyping by dilution, amplification, and sequencing" PNAS (April 2013) 110:5552-5557.	
	A66	KIRKNESS et al. "Sequencing of isolated sperm cells for direct haplotyping of a human genome," Genome Res (2013) 23:826-832.	
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	A72	LUNDIN, et al, "Hierarchical molecular tagging to resolve long continuous sequences by massively parallel sequencing", Sci Rep., 3:1186, 2003.	
	A73	MINER et al. "Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR" Nucleic Acids Res. 2004 Sep 30; 32(17):e135.	
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	A75	PCT/IB2010/002243, International Search Report and Written Opinion, mailed Feb. 9, 2011, 13pgs.	
	A76	REISNER, et al, "Single-molecule denaturation mapping of DNA in nanofluidic channels", Proc Natl Acad Sci U.S.A., 107: 13294-9, 2010.	
	A77	REPP et al. "Genotyping by Multiplex Polymerase Chain Reaction for Detection of Endemic Hepatitis B Virus Transmission" J Clinical Microbiology (1993) 31:1095-1102	
	A78	SCHWARTZ; et al., "Capturing native long-range contiguity by in situ library construction and optical sequencing", PNAS (Nov. 2012), 109(46)18749-18754. cited by applicant .	
	A79	TURNER, et al, "Assaying chromosomal inversions by single molecule haplotyping", Nat Methods., 3:439-445, 2006.	
	A80	TURNER, et al, "High-throughput haplotype determination over long distances by haplotype fusion PCR and ligation haplotyping", Nat Protoc., 4:1771-83, 2009.	
	A81	USHIJIMA et al, Detection and interpretation of altered methylation patterns in cancer cells, 2005, Nature reviews, 5, 223-231.	
Examiner Signature			Date Considered

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Substitute for form 1449A/PTO (Substitute PTO/SB/8A) INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application Number	16/194,047
				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	5	of	5	Attorney Docket Number	104371-5016-US10

NON PATENT LITERATURE DOCUMENTS				
Examiner Initials*	Cite No. ¹	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, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁶	
	A82	Van Nieuwerburgh, et al, "Illumina mate-paired DNA sequencing-library preparation using Cre-Lox recombination", Nucleic Acids Res., 40:1-8, 2012.		
	A83	WANG; et al., "Self-Formed Adaptor PCR: a Simple and Efficient Method for Chromosome Walking", Applied and Environmental Microbiology (Aug. 2007), 73(15):5048-5051.		
	A84	WISEMAN, R.W. et al. "Major histocompatibility complex genotyping with massively parallel pyrosequencing" Nature Medicine (Oct 11 2009) 15(11):1322-1326		
	A85	XIAO, et al, "Determination of haplotypes from single DNA molecules: a method for single-molecule barcoding", Hum Mutat., 28:913-21, 2007.		
	A86	YAN, PU et al. "Rapid on-step construction of hairpin RNA" Biochem and Biophys Res Comm (Jun 12 2009) 383(4):464-468		

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.¹ Applicant's unique citation designation number (optional).² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.⁶ Applicant is to place a check mark here if English language Translation is attached.

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

DB2/ 35620138.1

PARSE EX.1005.110

**TERMINAL DISCLAIMER TO OBTAIN A PROVISIONAL DOUBLE PATENTING
REJECTION OVER A PENDING "REFERENCE" APPLICATION**

Docket Number (Optional)

104371-5016-US10

In re Application of: 10X GENOMICS, INC.

Application No.: 16/194,047

Filed: November 16, 2018

For: METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

The applicant, 10X GENOMICS, INC., owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending **reference** Application Number 16/158,197, filed, October 11, 2018, as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the **reference** application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said **reference** application, "as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application," in the event that: any such patent granted on the pending **reference** application expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

Check either box 1 or 2 below, if appropriate.

1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

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

2. ☒ The undersigned is an attorney or agent of record. Reg. No. 58,729

<u>/Louis-Vu Nguyen/</u>	<u>January 17, 2019</u>
Signature	Date
<u>Louis-Vu Nguyen</u>	
Typed or printed name	
<u>Attorney of record</u>	<u>415.442.1000</u>
Title	Telephone Number

- ☒ Terminal disclaimer fee under 37 CFR 1.20(d) is included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.321. 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 12 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, 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.

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 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.

**TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING
REJECTION OVER A "PRIOR" PATENT**

Docket Number (Optional)

104371-5016-US10

In re Application of: 10X GENOMICS, INC.

Application No.: 16/194,047

Filed: November 16, 2018

For: METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

The applicant, 10X GENOMICS, INC., owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of **prior patent** No. 10,155,981 as the term of said **prior patent** is presently shortened by any terminal disclaimer. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

- expires for failure to pay a maintenance fee;
- is held unenforceable;
- is found invalid by a court of competent jurisdiction;
- is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
- has all claims canceled by a reexamination certificate;
- is reissued; or
- is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

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

2. ☒ The undersigned is an attorney or agent of record. Reg. No. 58,729

/Louis-Vu Nguyen/

Signature

January 17, 2019

Date

Louis-Vu Nguyen

Typed or printed name

Attorney of record

Title

415.442.1000

Telephone Number

- ☒ Terminal disclaimer fee under 37 CFR 1.20(d) included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.321. 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 12 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, 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.

Privacy Act Statement

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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.

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: 156830

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 assignment 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: 156830

OR

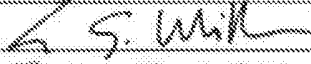
☐ Firm or individual name		
Address		
City	State	Zip
Country		
Telephone	Email	

Assignee name and address:
10x Genomics, Inc.
7082 Koll Center Parkway, Suite 401
Pleasanton, CA 94566

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 8-14-18
Name Eric S. Whitaker	Telephone
Title General Counsel	

This collection of information is required by 37 CFR 1.31, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public, which is to update (and by the USPTO to process) the file of a patent or reexamination proceeding. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 18 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, 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-6100 and select option 2.

Electronic Patent Application Fee Transmittal				
Application Number:		16194047		
Filing Date:		16-Nov-2018		
Title of Invention:		METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		
First Named Inventor/Applicant Name:		Sydney Brenner		
Filer:		Louis-Vu T. Nguyen/Sheila Gloss		
Attorney Docket Number:		104371-5016-US10		
Filed as Small 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:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
STATUTORY OR TERMINAL DISCLAIMER	2814	2	160	320
Total in USD (\$)				320

Electronic Acknowledgement Receipt

EFS ID:	34891395
Application Number:	16194047
International Application Number:	
Confirmation Number:	9126
Title of Invention:	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS
First Named Inventor/Applicant Name:	Sydney Brenner
Customer Number:	156830
Filer:	Louis-Vu T. Nguyen/Sheila Gloss
Filer Authorized By:	Louis-Vu T. Nguyen
Attorney Docket Number:	104371-5016-US10
Receipt Date:	17-JAN-2019
Filing Date:	16-NOV-2018
Time Stamp:	18:16:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$ 320
RAM confirmation Number	011819INTEFSW00018756500310
Deposit Account	
Authorized User	

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

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	104371-5016-US10_Stmt.pdf	83681	no	2
			b15374030a468d63dd5a711c3e6d3b9ebebc97ae		
Warnings:					
Information:					
2	Terminal Disclaimer Filed	104371-5016-US10_TD_App.pdf	241202	no	2
			0e206040f86288f455e154040e8f33ced0a09ce9		
Warnings:					
Information:					
3	Terminal Disclaimer Filed	104371-5016-US10_TD_Pat.pdf	156147	no	2
			746b5d118e7e0b91d253a97151cc1609f66d06a2		
Warnings:					
Information:					
4	Power of Attorney	10x_ExePOA.pdf	294608	no	1
			96738d746af9be35be3363b824e4bee772089c0e		
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30475	no	2
			1c582076bbabbf5b5d142db312f59e7bbea8f5aa		
Warnings:					
Information:					
Total Files Size (in bytes):			806113		

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.

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

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.

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 displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: 10X GENOMICS, INC.

Application No./Patent No.: 16/194,047

Filed/Issue Date: November 16, 2018

Titled: METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

10X 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: Inventors To: Population Genetics Technologies Ltd.

The document was recorded in the United States Patent and Trademark Office at
Reel 047579, Frame 0304, or for which a copy thereof is attached.

2. From: Population Genetics Technologies Ltd. To: 10X GENOMICS, INC.

The document was recorded in the United States Patent and Trademark Office at
Reel 047579, Frame 0515, or for which a copy thereof is attached.

[Page 1 of 2]

This collection of information is required by 37 CFR 3.73(b). 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 12 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, 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.

PARSE EX.1005.122

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.

/Louis-Vu Nguyen/

Signature

Louis-Vu Nguyen

Printed or Typed Name

January 17, 2019

Date

58,729

Title or Registration Number



UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/194,047	11/16/2018	Sydney Brenner	104371-5016-US10

CONFIRMATION NO. 9126

POA ACCEPTANCE LETTER

156830
MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics
One Market, Spear Street Tower, Suite 2800
San Francisco, CA 94105



Date Mailed: 01/24/2019

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 01/17/2019.

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.

/yfeferra/

Application Number * 16/194,047 *	Application/Control No. 16/194,047	Applicant(s)/Patent under Reexamination Brenner et al.	
	Examiner DINES, KARLA A	Art Unit 1639	
Document Code - DISQ		Internal Document - DO NOT MAIL	

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed: <u>17 January 2019</u>	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:
/JEAN PROCTOR/ Technology Center: OPLC Telephone: (571)272-1040 2 td's

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Sydney Brenner et al.

Application No.: **16/194,047**

Filed: **November 16, 2018**

For: **METHODS FOR ANALYZING
NUCLEIC ACIDS FROM SINGLE
CELLS**

Confirmation No.: **9126**

Examiner: **Karla A. Dines**

Art Unit: **1639**

PRELIMINARY AMENDMENT

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

Commissioner:

Please enter the following amendments and remarks:

Amendments to the Specification being on page 2 of this paper.

Remarks begin on page 3 of this paper.

Amendments to the Specification:

Please insert the following header and paragraph immediately after the title on Page 1:

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. Application No. 15/677,957, filed August 15, 2017, now Patent No. 10,155,981, which is a continuation of U.S. Application No. 14/792,094, filed July 6, 2015, which is a continuation of U.S. Application No. 14/172,694, filed February 4, 2014, now Patent No. 9,102,980, which is a continuation of U.S. Application No. 14/021,790, filed September 9, 2013, now Patent No. 8,679,756, which is a continuation of U.S. Application No. 13/859,450, filed April 9, 2013, now Patent No. 8,563,274, which is a continuation of U.S. Application No. 13/622,872, filed September 19, 2012, which is a continuation of U.S. Application No. 13/387,343, filed February 15, 2012, now Patent No. 8,298,767, which is a §371 National Phase Application of PCT/IB2010/002243, filed August 13, 2010, which claims priority to U.S. Provisional Application No. 61/235,595, filed August 20, 2009 and U.S. Provisional Application No. 61/288,792, filed December 21, 2009; all of which are herein incorporated by reference.

REMARKS

The Specification has been amended to include the cross reference paragraph. Applicants note that the priority claim information in the cross reference paragraph was included on the Application Data Sheet filed November 16, 2018 and is reflected in the Filing Receipt issued December 11, 2018.

This amendment includes no new matter. Entry of the amendment is respectfully requested.

Except for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any necessary fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17, which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-0310 (Order No.: 104371-5016-US10).

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested.

Respectfully submitted,

MORGAN, LEWIS & BOCKIUS LLP

Dated: January 30, 2019

Customer No.: 43850

Morgan, Lewis & Bockius LLP
One Market, Spear Street Tower

San Francisco, CA 94105

Telephone: (415) 442-1000

Facsimile: (415) 442-1001

By: /Louis-Vu Nguyen /

Louis-Vu Nguyen, Reg. No. 58,723, for
Gargi Talukder, Reg. No. 61,368

Electronic Acknowledgement Receipt

EFS ID:	35011888
Application Number:	16194047
International Application Number:	
Confirmation Number:	9126
Title of Invention:	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS
First Named Inventor/Applicant Name:	Sydney Brenner
Customer Number:	156830
Filer:	Louis-Vu T. Nguyen/Sheila Gloss
Filer Authorized By:	Louis-Vu T. Nguyen
Attorney Docket Number:	104371-5016-US10
Receipt Date:	30-JAN-2019
Filing Date:	16-NOV-2018
Time Stamp:	19:17:19
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		104371_5016US10_Amendment.pdf	133990 309c30b326fe260ed043387596e4459d28b a3c81	yes	3

Multipart Description/PDF files in .zip description			
Document Description		Start	End
Preliminary Amendment		1	1
Specification		2	2
Applicant Arguments/Remarks Made in an Amendment		3	3
Warnings:			
Information:			
Total Files Size (in bytes):		133990	
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.			



UNITED STATES PATENT AND TRADEMARK OFFICE

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

NOTICE OF ALLOWANCE AND FEE(S) DUE

156830 7590 02/01/2019
MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics
One Market, Spear Street Tower, Suite 2800
San Francisco, CA 94105

EXAMINER	
DINES, KARLA A	
ART UNIT	PAPER NUMBER
1639	

DATE MAILED: 02/01/2019

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/194,047	11/16/2018	Sydney Brenner	104371-5016-US10	9126

TITLE OF INVENTION: METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$500	\$0.00	\$0.00	\$500	05/01/2019

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 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity 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 and an extra copy of the form should be submitted. 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 including the Patent, advance orders and notification of maintenance fees 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.

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

156830 7590 02/01/2019
MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics
One Market, Spear Street Tower, Suite 2800
San Francisco, CA 94105

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.

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/194,047	11/16/2018	Sydney Brenner	104371-5016-US10	9126

TITLE OF INVENTION: METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$500	\$0.00	\$0.00	\$500	05/01/2019

EXAMINER	ART UNIT	CLASS-SUBCLASS
DINES, KARLA A	1639	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/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, 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) ☐ Advance Order - # of Copies _____

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

- ☐ Electronic Payment via 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. _____



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/194,047	11/16/2018	Sydney Brenner	104371-5016-US10	9126
156830	7590	02/01/2019	EXAMINER	
MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics One Market, Spear Street Tower, Suite 2800 San Francisco, CA 94105			DINES, KARLA A	
			ART UNIT	PAPER NUMBER
			1639	
DATE MAILED: 02/01/2019				

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); (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. 16/194,047	Applicant(s) Brenner et al.	
	Examiner KARLA A DINES	Art Unit 1639	AIA Status No

-- 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 communication filed 12/20/2018.
☐ 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 1-5 and 16-36. 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)	5. ☐ Examiner's Amendment/Comment
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 1/14/2019.	6. ☒ Examiner's Statement of Reasons for Allowance
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.	7. ☐ Other _____.
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date. 1/15/2019.	

/KARLA A DINES/ Primary Examiner, Art Unit 1639	
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Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Status of the Claims

Claims 6-15 are cancelled.

Claims 1-5 and 16-36 are currently pending and examined on the merits.

Information Disclosure Statement

The information disclosure statement filed 1/14/2019 is acknowledged. A signed copy of the corresponding 1449 form has been included with this Office action.

Drawings

The drawings filed on 11/16/2018 are received and accepted.

Reasons for Allowance

The following is an examiner's statement of reasons for allowance:

A terminal disclaimer was filed on 1/17/2019 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of US patent No: 10,155,981.

Conclusion

Claims 1-5 and 16-36 are allowed.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to KARLA A DINES whose telephone number is (571)272-6837. The examiner can normally be reached on Monday-Friday 7am-4pm.

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, Heather Calamita can be reached on 571-272-2876. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should

you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/KARLA A DINES/
Primary Examiner, Art Unit 1639

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record

A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) 1.133 Interviews

Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.

All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiners responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicants correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

- 1) A brief description of the nature of any exhibit shown or any demonstration conducted,-
- 2) an identification of the claims discussed,
- 3) an identification of the specific prior art discussed,
- 4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
- 5) a brief identification of the general thrust of the principal arguments presented to the examiner,
(The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
- 6) a general indication of any other pertinent matters discussed, and
- 7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicants record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiners version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, Interview Record OK on the paper recording the substance of the interview along with the date and the examiners initials.

Issue Classification 	Application/Control No. 16/194,047	Applicant(s)/Patent Under Reexamination Brenner et al.
	Examiner KARLA A DINES	Art Unit 1639

CPC						
Symbol					Type	Version
C12Q	/	1	/	6874	F	2013-01-01
C12Q	/	1	/	686	I	2013-01-01
C12Q	/	1	/	6806	I	2013-01-01
C12N	/	15	/	1065	I	2013-01-01
C12Q	/	1	/	6855	I	2013-01-01

CPC Combination Sets					
Symbol				Type	Set
	/		/		

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	26	
/KARLA A DINES/ Primary Examiner, Art Unit 1639	27 January 2019	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

Issue Classification 	Application/Control No. 16/194,047	Applicant(s)/Patent Under Reexamination Brenner et al.	
	Examiner KARLA A DINES	Art Unit 1639	

INTERNATIONAL CLASSIFICATION			
CLAIMED			
C12Q		1	6874
NON-CLAIMED			

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS
506	4

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

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	26	
/KARLA A DINES/ Primary Examiner, Art Unit 1639	27 January 2019	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

Issue Classification 	Application/Control No. 16/194,047	Applicant(s)/Patent Under Reexamination Brenner et al.
	Examiner KARLA A DINES	Art Unit 1639

☒ 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
1	1	10	20	19	29										
2	2	11	21	20	30										
3	3	12	22	21	31										
4	4	13	23	22	32										
5	5	14	24	23	33										
6	16	15	25	24	34										
7	17	16	26	25	35										
8	18	17	27	26	36										
9	19	18	28												

NONE	Total Claims Allowed:	
(Assistant Examiner)	(Date)	26
/KARLA A DINES/ Primary Examiner, Art Unit 1639	27 January 2019	O.G. Print Claim(s)
(Primary Examiner)	(Date)	1
		O.G. Print Figure
		none

<i>Search Notes</i> 	Application/Control No. 16/194,047	Applicant(s)/Patent Under Reexamination Brenner et al.
	Examiner KARLA A DINES	Art Unit 1639

CPC - Searched*		
Symbol	Date	Examiner
C12Q1/6874	01/27/2019	kd

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner
506	004	01/27/2019	kd

* 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
EAST and PALM searches	01/27/2019	kd
EAST and PubMed searches	01/27/2019	kd
C12Q1/6874	01/27/2019	kd
506/004	01/27/2019	kd

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
506	004	01/27/2019	kd
C12Q1	6874	01/27/2019	kd

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PATENT

Application No. 16/194,047
Attorney Docket No. 104371-5016-US10**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

First Named Inventor:

Sidney BRENNERApplication No.: **16/194,047**Filed: **November 16, 2018**For: **Methods for Analyzing Nucleic Acids
from Single Cells**Conf. No.: **9126**Art Unit: **1639**Examiner: **Karla A. Dines**Docket No.: **104371-5016-US10***Certificate of Transmission (37 C.F.R. § 1.8)**I hereby certify that this correspondence is being transmitted to the
U.S. Patent and Trademark Office via the Office electronic filing
system (EFS-Web) to the USPTO on January 14, 2019.*/Sheila R. Gloss/*Sheila R. Gloss*Mail Stop AMENDMENT
Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450**INFORMATION DISCLOSURE STATEMENT**

Commissioner:

This Information Disclosure Statement is hereby submitted in accordance with 37 C.F.R. 1.98 and pursuant to Applicant's continuing duty under 37 C.F.R. 1.56 to bring any information which may be material to patentability of this application to the Examiner's attention.

Applicant makes no representation that a search has been conducted by the Applicant, or that there is not possibly more relevant art. 37 C.F.R. § 1.97(g). Applicant also makes no representation that the information submitted herewith is in fact material to patentability. The filing of this Information Disclosure Statement shall not be construed as an admission against interest in any manner. 37 C.F.R. § 1.97(h).

The Examiner's attention is directed to the reference(s) cited on the accompanying substitute for forms 1449A/PTO and PTO/SB/08. Except for U.S. Patents and U.S. Patent Application Publications, *see* 37 C.F.R. § 1.98(a)(2)(i), copies of the cited references are enclosed, unless otherwise noted below.

PATENT

Application No. 16/194,047
Attorney Docket No. 104371-5016-US10

This Information Disclosure Statement is being filed within three months of the filing date of a national application other than a continued prosecution application, within three months of the date of entry of a national stage, before the mailing of a first Office action on the merits, or before the mailing of a first Office action after the filing of a request for continued examination. 37 C.F.R. § 1.97(b). No fee is required.

This Application relies on the earlier filing date of prior Application No. 15/667957, filed August 15, 2017; Application No. 14/792,094, filed July 6, 2015; Application No. 14/021,790, filed September 9, 2013; Application No. 13/859,450, filed April 9, 2013; Application No. 13/622,872, filed September 19, 2012; and Application No. 13/387,343, filed February 12, 2012, under 35 U.S.C. § 120. It is understood that the Examiner will consider information that was submitted to or cited by the Office in such prior applications. M.P.E.P. § 609.02(A)(2). Copies of documents previously submitted to or cited by the Office in the prior application are not required to be provided in this application and thus are not attached. 37 C.F.R. § 1.98(d).

The Commissioner is hereby authorized to charge any underpayment of the following fees associated with this communication, including any necessary fees for the submission of an information disclosure statement under 37 CFR § 1.17(p) or for the extension of time, or credit any overpayment to Deposit Account No. 50-0310.

Respectfully submitted,

Date: January 14, 2019
MORGAN LEWIS & BOCKIUS LLP
One Market, Spear Street Tower
San Francisco, California 94105
Tel: 415.442.1000
Fax: 415.442.1001

By: /Louis-Vu Nguyen/
Louis-Vu Nguyen, Reg. No. 58,729, for
Gargi Talukder, Reg. No. 61,368
Filed Under 37 C.F.R. § 1.34

Substitute for form 1449A/PTO (Substitute PTO/SB/8A) INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application Number	16/194,047
				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	1	of	5	Attorney Docket Number	104371-5016-US10

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	U.S. Patent Document Number-Kind Code ² (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A1	US-2003/0022231-A1	01-30-2003	WANGH	
	A2	US-2003/0050453-A1	03-13-2003	SORGE	
	A3	US-2003/0207300-A1	11-06-2003	MATRAY et al.	
	A4	US-2004/0224331	11-11-2004	CANTOR et al.	
	A5	US-2005/0250147-A1	11-10-2005	MACEVICZ	
	A6	US-2006/0008799	01-12-2006	CAI et al.	
	A7	US 2006/0177832 A1	08-10-2006	BRENNER	
	A8	US 2006/0177833 A1	08-10-2006	BRENNER	
	A9	US-2007/0020640	01-25-2007	MCCLOSKEY et al.	
	A10	US-2007/0072208-A1	03-29-2007	DRMANAC	
	A11	US-2007/0238113-A1	11-10-2007	KANDA et al.	
	A12	US-2007/0259357-A1	11-08-2007	BRENNER	
	A13	US-2008/0274458-A1	11-06-2008	LATHAM et al.	
	A14	US-2009/0105959-A1	04-23-2009	BRAVERMAN	
	A15	US-2009/0155780	06-18-2009	XIAO et al.	
	A16	US-2010/0086914	04-08-2010	BENTLEY et al.	
	A17	US-2010/0113296	05-06-2010	MYERSON	
	A18	US-2010/0120098	05-13-2010	GRUNENWALD et al.	
	A19	US-2011/0287435	11-24-2011	GRUNENWALD et al.	
	A20	US-2012/0208724	08-16-2012	STEEMERS	
	A21	US-2012/0316074-A1	12-13-2012	SAXONOV	
	A22	US-2013/0059310	03-07-2013	BRENNER et al.	
	A23	US-5846719-A	12-08-1998	BRENNER et al.	
	A24	US-6677121-B2	01-13-2004	LIZARDI et al.	
	A25	US-6723513	04-20-2004	LEXOW	
	A26	US-7217522-B2	05-15-2007	BRENNER	
	A27	US 7329493 B2	02-12-2008	CHOU et al.	

Examiner Signature		Date Considered	
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 809. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.¹ Applicant's unique citation designation number (optional).² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.⁶ Applicant is to place a check mark here if English language Translation is attached.

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

DB2/ 35620138.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.A.D/

PARSE EX.1005.147

Substitute for form 1449A/PTO (Substitute PTO/SB/8A) INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application Number	16/194,047
				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	2	of	5	Attorney Docket Number	104371-5016-US10

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	U.S. Patent Document Number-Kind Code ² (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A28	US-7393665-B2	07-01-2008	BRENNER	
	A29	US-7537897-B2	05-26-2009	BRENNER et al.	
	A30	US-7544473	06-09-2009	BRENNER	
	A31	US-7700325	04-20-2010	CANTOR et al.	
	A32	US-8101346	01-24-2012	TAKAHAMA	
	A33	US-8168385-B2	05-01-2012	BRENNER	
	A34	US-8298767-B2	10-30-2012	BRENNER et al.	
	A35	US-8318433-B2	11-27-2012	BRENNER	
	A36	US-8318460-B2	11-27-2012	CANTOR et al.	
	A37	US-8563274-B2	10-22-2013	BRENNER	
	A38	US-8679756-B1	03-25-2014	BRENNER et al.	
	A39	US-9085798		CHEE	
	A40	US-9102980-B2	08-11-2015	BRENNER	
	A41	US-9290808-B2	03-22-2016	FODOR	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document Country Code ² Number ⁴ Kind Code ⁵ (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T ⁶
	A42	EP-1672064-A1	06-21-2006	Riken		
	A43	EP-1841879-A2	10-10-2007	Compass Genetics, LLC		
	A44	WO-2001090418-A1	11-29-2001	The Regents of the University of California		
	A45	WO-2003062462-A2	07-31-2003	Bosnes		
	A46	WO-2004061083-A2	07-22-2004	Amgen Inc.		
	A47	WO-2004065617-A2	08-05-2004	The Trustees of Boston University		
	A48	WO-2007018601-A1	02-15-2007	Rubicon Genomics, Inc.		
	A49	WO 2007147079 A2	12-21-2007	Living Microsystems, Inc. et al.		
	A50	WO-2008061193-A3	11-13-2008	Biospherex LLC et al.		

Examiner Signature		Date Considered	
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Substitute for form 1449A/PTO (Substitute PTO/SB/8A) INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application Number	16/194,047
				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	3	of	5	Attorney Docket Number	104371-5016-US10

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document Country Code ² Number ⁴ Kind Code ⁵ (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T ⁶
	A51	WO-2008150432-A1	12-11-2008	454 Life Sciences Corp		
	A52	WO 2010117620 A3	10-14-2010	Illumina, Inc.		
	A53	WO-2012148497-A2	11-01-2012	Robinson et al.		
	A54	WO-2013036929-A1	03-14-2013	The Board of Trustees of the Leland Stanford Jr University		

NON PATENT LITERATURE DOCUMENTS						
Examiner Initials*	Cite No. ¹	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, page(s), volume-issue number(s), publisher, city and/or country where published.				T ⁶
	A55	"Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry Basic MID Set" Genome Sequencer FLX System, Technnical Bulletin 004-2009, (Apr 1 2009) pp 1-7. URL:http://454.com/downloads/my454/documentation/technical-bulletins/TCB-09004 Using MultiplexIdentifierAdaptorsForTheGSFLXTitaniumSeriesChemistry-BasicMIDSet.pdf				
	A56	"Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry Extended MID Set" Genome Sequencer FLX System, Technnical Bulletin 005-2009, (Apr 1 2009) pp 1-7. URL:http://454.com/downloads/my454/documentation/technical-bulletins/TCB-09005 UsingMultiplexIdentifierAdaptorsForTheGSFLXTitaniumChemistry-ExtendedMIDSet.pdf				
	A57	AHERN, "Biochemical, Reagents Kits Offer Scientists Good Return on Investment" The Scientist (1995) 9(15):1-7				
	A58	BOULANGER, et al, "Massively parallel haplotyping on microscopic beads for the high-throughput phase analysis of single molecules", PLoS One, vol. 7:1-10, 2012.				
	A59	BRENNER, S. et al. "In vitro cloning of complex mixtures of DNA on microbeads: Physical separation of differentially expressed cDNAs" PNAS 2000 Feb. 15; 97(4):1665-70.				
	A60	CASBON, et al, "Reflex: intramolecular barcoding of long-range PCR products for sequencing multiple pooled DNAs", Nucleic Acids Res., pp. 1-6, 2013.				
	A61	FAN, et al, "Whole-genome molecular haplotyping of single cells", Nat Biotechnol., 29: 51-59, 2011.				
	A62	HIATT, et al, "Parallel, tag-directed assembly of locally derived short sequence reads", Nat Methods., 7:119-122, 2010.				
	A63	HUG, H. et al. "Measurement of the number of molecules of a single mRNA species in a complex mRNA preparation" J Theor Biol. 2003 Apr 21; 221(4):615-24.				
	A64	IMBURGIO, et al, "Studies of promoter recognition and start site selection by T7 RNA polymerase using a comprehensive collection of promoter variants", Biochemistry., 39:10419-30, 2000.				

Examiner Signature		Date Considered	
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 809. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.¹ Applicant's unique citation designation number (optional).² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.⁶ Applicant is to place a check mark here if English language Translation is attached.

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Substitute for form 1449A/PTO (Substitute PTO/SB/8A) INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application Number	16/194,047
				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	4	of	5	Attorney Docket Number	104371-5016-US10

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	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, page(s), volume-issue number(s), publisher, city and/or country where published.	TS
	A65	KAPER, F. et al. "Whole-genome haplotyping by dilution, amplification, and sequencing" PNAS (April 2013) 110:5552-5557.	
	A66	KIRKNESS et al. "Sequencing of isolated sperm cells for direct haplotyping of a human genome," Genome Res (2013) 23:826-832.	
	A67	KITZMAN et al. "Haplotype-resolved genome sequencing of a Gujarati Indian individual." Nat Biotechnol (2011) 29:59-63.	
	A68	KONAREWA, et al, "96-plex molecular barcoding for the Illumina Genome Analyzer", Methods Mol Biol., 733:279-98, 2011.	
	A69	KOZAREWA, et al. "Amplification-free Illumina sequencing-library preparation facilitates improved mapping and assembly of GC-biased genomes", Nat Methods., 6: 291-5, 2009.	
	A70	KWOK, et al, "Single-molecule analysis for molecular haplotyping", Hum Mutat., 23:442-6, 2004.	
	A71	LAIRD et al, Hairpin-bisulfite PCR: Assessing epigenetic methylation patterns on complementary strands of individual DNA molecules, 2004, PNAS, 101, 204-209.	
	A72	LUNDIN, et al, "Hierarchical molecular tagging to resolve long continuous sequences by massively parallel sequencing", Sci Rep., 3:1186, 2003.	
	A73	MINER et al. "Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR" Nucleic Acids Res. 2004 Sep 30; 32(17):e135.	
	A74	OYOLA, et al, "Optimizing Illumina next-generation sequencing library preparation for extremely AT-biased genomes", BMC Genomics.,13:1, 2012. cited by applicant .	
	A75	PCT/IB2010/002243, International Search Report and Written Opinion, mailed Feb. 9, 2011, 13pgs.	
	A76	REISNER, et al, "Single-molecule denaturation mapping of DNA in nanofluidic channels", Proc Natl Acad Sci U.S.A., 107: 13294-9, 2010.	
	A77	REPP et al. "Genotyping by Multiplex Polymerase Chain Reaction for Detection of Endemic Hepatitis B Virus Transmission" J Clinical Microbiology (1993) 31:1095-1102	
	A78	SCHWARTZ; et al., "Capturing native long-range contiguity by in situ library construction and optical sequencing", PNAS (Nov. 2012), 109(46)18749-18754. cited by applicant .	
	A79	TURNER, et al, "Assaying chromosomal inversions by single molecule haplotyping", Nat Methods., 3:439-445, 2006.	
	A80	TURNER, et al, "High-throughput haplotype determination over long distances by haplotype fusion PCR and ligation haplotyping", Nat Protoc., 4:1771-83, 2009.	
	A81	USHIJIMA et al, Detection and interpretation of altered methylation patterns in cancer cells, 2005, Nature reviews, 5, 223-231.	
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.¹ Applicant's unique citation designation number (optional).² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.⁶ Applicant is to place a check mark here if English language Translation is attached.

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

DB2/ 35620138.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.A.D/

PARSE EX.1005.150

Substitute for form 1449A/PTO (Substitute PTO/SB/8A) INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application Number	16/194,047
				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	5	of	5	Attorney Docket Number	104371-5016-US10

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	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, page(s), volume-issue number(s), publisher, city and/or country where published.	†6
	A82	Van Nieuwerburgh, et al, "Illumina mate-paired DNA sequencing-library preparation using Cre-Lox recombination", Nucleic Acids Res., 40:1-8, 2012.	
	A83	WANG; et al., "Self-Formed Adaptor PCR: a Simple and Efficient Method for Chromosome Walking", Applied and Environmental Microbiology (Aug. 2007), 73(15):5048-5051.	
	A84	WISEMAN, R.W. et al. "Major histocompatibility complex genotyping with massively parallel pyrosequencing" Nature Medicine (Oct 11 2009) 15(11):1322-1326	
	A85	XIAO, et al, "Determination of haplotypes from single DNA molecules: a method for single-molecule barcoding", Hum Mutat., 28:913-21, 2007.	
	A86	YAN, PU et al. "Rapid on-step construction of hairpin RNA" Biochem and Biophys Res Comm (Jun 12 2009) 383(4):464-468	

Examiner Signature	/KARLA A DINES/	Date Considered	01/15/2019
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.¹ Applicant's unique citation designation number (optional).² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.⁶ Applicant is to place a check mark here if English language Translation is attached.

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DB2/ 35620138.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.A.D/

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	0	(count near polynucleotides near sample)and cell and tag	USPAT	OR	OFF	2019/01/27 16:12
S2	0	count near polynucleotides near sample	USPAT	OR	OFF	2019/01/27 16:14
S3	1	count near polynucleotides	USPAT	OR	OFF	2019/01/27 16:15
S4	1	count near polynucleotides	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 16:27
S5	63	count near number near fragments	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 16:27
S6	0	S5 and (polynucleotides same cells)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 16:28
S7	0	S5 and polynucleotides and cells	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 16:28
S8	4	"10155981".pn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 16:30
S9	94	"10x genomics".asn.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 16:50
S10	447	brenner-sydney.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 17:19
S11	34	S10 and (count\$3 same (polynucleotides or "nucleic acids" or rna or mrna))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 17:20
S12	61	mikawa-gi.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 17:57
S13	3	S12 and (count\$3 same (polynucleotides or "nucleic acids" or rna or mrna))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 17:57
S14	211	osborne-robert.in.	US-PGPUB; USPAT;	OR	OFF	2019/01/27

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			17:58
S15	20	S14 and (count\$3 same (polynucleotides or "nucleic acids" or rna or mrna))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 17:58
S16	48	slatter-andrew.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 17:59
S17	2	S16 and (count\$3 same (polynucleotides or "nucleic acids" or rna or mrna))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 17:59
S18	0	506/004.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 17:59
S19	1215	506/4.ccls.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:00
S20	109	S19 and (count\$3 same (polynucleotides or "nucleic acids" or rna or mrna))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:00
S21	67	S20 and tags	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:09
S22	57	S20 and tags and cells	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:10
S23	20	S20 and tags and "single cells"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:10
S24	230	"I24" and (count\$3 same (polynucleotides or "nucleic acids" or rna or mrna))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:12
S25	9972	c12q1/6874.cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:15
S26	607	S25 and (count\$3 same (polynucleotides or "nucleic acids" or rna or mrna))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:15
S27	388	S26 and tags	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:16
S28	387	S26 and tags and sequence	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:16

S29	388	S26 and tags and sequenc\$3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/01/27 18:16
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EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S30	196	506/4.ccls.	USPAT	OR	OFF	2019/01/27 18:11
S31	69	"I24" and (count\$3 same (polynucleotides or "nucleic acids" or rna or mrna))	USPAT	OR	OFF	2019/01/27 18:12
S32	1278	c12q1/6874.cpc.	USPAT	OR	OFF	2019/01/27 18:14
S33	177	S32 and (count\$3 same (polynucleotides or "nucleic acids" or rna or mrna))	USPAT	OR	OFF	2019/01/27 18:14

1/ 27/ 2019 10:38:46 PM

C:\Users\kdines\Documents\EAST\Workspaces\16194047.wsp

Bibliographic Data

Application No: 16/194,047

Foreign Priority claimed: ☐ Yes ☒ No

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

Verified and Acknowledged:

/KARLA A DINES/

Examiner's Signature

Initials

Title:

METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/16/2018	506	1639	104371-5016-US10
RULE			

APPLICANTS

10X Genomics, Inc., Pleasanton, CA, UNITED STATES

INVENTORS

Sydney Brenner Ely, UNITED KINGDOM

Gi Mikawa Great Shelford, UNITED KINGDOM

Robert Osborne Great Chesterford, UNITED KINGDOM

Andrew Slatter London, UNITED KINGDOM

CONTINUING DATA

This application is a CON of 15677957 08/15/2017 PAT 10155981

15677957 is a CON of 14792094 07/06/2015ABN

14792094 is a CON of 14172694 02/04/2014 PAT 9102980

14172694 is a CON of 14021790 09/09/2013 PAT 8679756

14021790 is a CON of 13859450 04/09/2013 PAT 8563274

13859450 is a CON of 13622872 09/19/2012ABN

13622872 is a CON of 13387343 02/15/2012 PAT 8298767

13387343 is a 371 of PCT/IB2010/002243 08/13/2010

PCT/IB2010/002243 has PRO of 61288792 12/21/2009

PCT/IB2010/002243 has PRO of 61235595 08/20/2009

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

12/07/2018

** SMALL ENTITY **

STATE OR COUNTRY

UNITED KINGDOM

ADDRESS

MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics

One Market, Spear Street Tower, Suite 2800

San Francisco, CA 94105

UNITED STATES

FILING FEE RECEIVED

\$2,855

#5	Add	Search Slater, Andrew[Author]	41
#5	Add	Search osborne, robert[Author]	33
#4	Add	Search (#1) AND #3	9
#3	Add	Search mikawa[Author]	1038
#2	Add	Search mikawa, gi[Author]	9
#1	Add	Search brenner, sydney[Author]	101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Sydney Brenner et al.

Application No.: **16/194,047**

Filed: **November 16, 2018**

For: **METHODS FOR ANALYZING
NUCLEIC ACIDS FROM SINGLE
CELLS**

Confirmation No.: **9126**

Examiner: **Karla A. Dines**

Art Unit: **1639**

**STATEMENT OF SUBSTANCE OF
INTERVIEW**

Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicants thank Examiner Dines for her time during the telephonic interview conducted on January 15, 2019 with Applicants' representatives Dr. Gargi Talukder and Louis Nguyen. During the interview, support for the claims was discussed.

Respectfully submitted,

Dated: February 4, 2019

Customer No.: 43850

Morgan, Lewis & Bockius LLP

One Market, Spear Street Tower

San Francisco, CA 94105

Telephone: (415) 442-1000

Facsimile: (415) 442-1001

By: /Gargi Talukder/

Gargi Talukder, Reg. No. 61,368

PART B - FEE(S) TRANSMITTAL

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

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P.O. Box 1450
Alexandria, Virginia 22313-1450

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

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156830 7590 02/01/2019
MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics
One Market, Spear Street Tower, Suite 2800
San Francisco, CA 94105

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Sheila R. Gloss	(Typed or printed name)
<i>Sheila R. Gloss</i>	(Signature)
February 4, 2019	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/194,047	11/16/2018	Sydney Brenner	104371-5016-US10	9126

TITLE OF INVENTION: METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$500	\$0.00	\$0.00	\$500	05/01/2019

EXAMINER	ART UNIT	CLASS-SUBCLASS
DINES, KARLA A	1639	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/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 Morgan, Lewis & Bockius LLP
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

10X GENOMICS, INC.

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

Pleasanton, CA

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) ☐ Advance Order - # of Copies _____

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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

Gargi Talukder

Date

February 4, 2019

Typed or printed name

Gargi Talukder

Registration No.

61,368

Electronic Patent Application Fee Transmittal				
Application Number:		16194047		
Filing Date:		16-Nov-2018		
Title of Invention:		METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS		
First Named Inventor/Applicant Name:		Sydney Brenner		
Filer:		Gargi Talukder/Sheila Gloss		
Attorney Docket Number:		104371-5016-US10		
Filed as Small 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	2501	1	500	500

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				500

Electronic Acknowledgement Receipt

EFS ID:	35053480
Application Number:	16194047
International Application Number:	
Confirmation Number:	9126
Title of Invention:	METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS
First Named Inventor/Applicant Name:	Sydney Brenner
Customer Number:	156830
Filer:	Gargi Talukder/Sheila Gloss
Filer Authorized By:	Gargi Talukder
Attorney Docket Number:	104371-5016-US10
Receipt Date:	04-FEB-2019
Filing Date:	16-NOV-2018
Time Stamp:	18:12:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$ 500
RAM confirmation Number	020519INTEFSW00005340500310
Deposit Account	
Authorized User	

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

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant summary of interview with examiner	104371-5016-US10_IntSummary.pdf	124260	no	1
			1ac19948017d0bc20a7374c5552abc1c6319f446		
Warnings:					
Information:					
2	Issue Fee Payment (PTO-85B)	104371-5016-US10_IssueFeeTrans.pdf	141055	no	1
			faad83a203060c30d6db5b9081d184226fa3c3c1		
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30441	no	2
			eefc67db72aa291d54f574083d979ccd067a3574		
Warnings:					
Information:					
Total Files Size (in bytes):			295756		
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.					

Substitute for form 1449A/PTO (Substitute PTO/SB/8A) INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application Number	16/194,047
				Filing Date	November 16, 2018
				First Named Inventor	Sidney BRENNER
				Art Unit	1639
				Examiner Name	Karla A. Dines
Sheet	2	of	5	Attorney Docket Number	104371-5016-US10

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No. ¹	U.S. Patent Document Number-Kind Code ² (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A28	US-7393665-B2	07-01-2008	BRENNER	
	A29	US-7537897-B2	05-26-2009	BRENNER et al.	
	A30	US-7544473	06-09-2009	BRENNER	
	A31	US-7700325	04-20-2010	CANTOR et al.	
	A32	US-8101346	01-24-2012	TAKAHAMA	
	A33	US-8168385-B2	05-01-2012	BRENNER	
	A34	US-8298767-B2	10-30-2012	BRENNER et al.	
	A35	US-8318433-B2	11-27-2012	BRENNER	
	A36	US-8318460-B2	11-27-2012	CANTOR et al.	
	A37	US-8563274-B2	10-22-2013	BRENNER	
	A38	US-8679756-B1	03-25-2014	BRENNER et al.	
	A39	US-9085798	07/2015	CHEE	
	A40	US-9102980-B2	08-11-2015	BRENNER	
	A41	US-9290808-B2	03-22-2016	FODOR	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No. ¹	Foreign Patent Document Country Code ² Number ⁴ Kind Code ⁵ (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T ⁶
	A42	EP-1672064-A1	06-21-2006	Riken		
	A43	EP-1841879-A2	10-10-2007	Compass Genetics, LLC		
	A44	WO-2001090418-A1	11-29-2001	The Regents of the University of California		
	A45	WO-2003062462-A2	07-31-2003	Bosnes		
	A46	WO-2004061083-A2	07-22-2004	Amgen Inc.		
	A47	WO-2004065617-A2	08-05-2004	The Trustees of Boston University		
	A48	WO-2007018601-A1	02-15-2007	Rubicon Genomics, Inc.		
	A49	WO 2007147079 A2	12-21-2007	Living Microsystems, Inc. et al.		
	A50	WO-2008061193-A3	11-13-2008	Biospherex LLC et al.		

Examiner Signature		Date Considered	
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 809. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.¹ Applicant's unique citation designation number (optional).² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.⁶ Applicant is to place a check mark here if English language Translation is attached.

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

DB2/ 35620138.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.A.D/

PARSE EX.1005.165



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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/194,047	03/26/2019	10240197	104371-5016-US10	9126

156830 7590 03/06/2019
MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics
One Market, Spear Street Tower, Suite 2800
San Francisco, CA 94105

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

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 0 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 Data Management (ODM) at (571)-272-4200.

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

Sydney Brenner, Ely, UNITED KINGDOM;
10X Genomics, Inc., Pleasanton, CA;
Gi Mikawa, Great Shelford, UNITED KINGDOM;
Robert Osborne, Great Chesterford, UNITED KINGDOM;
Andrew Slatter, London, UNITED KINGDOM;

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.



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www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/194,047	11/16/2018	Sydney Brenner	104371-5016-US10

CONFIRMATION NO. 9126

156830

MORGAN, LEWIS & BOCKIUS LLP - 10X Genomics
One Market, Spear Street Tower, Suite 2800
San Francisco, CA 94105

PUBLICATION NOTICE



OC000000106761870

Title:METHODS FOR ANALYZING NUCLEIC ACIDS FROM SINGLE CELLS

Publication No.US-2019-0085392-A1

Publication Date:03/21/2019

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 publicly 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

AO 120 (Rev. 08/10)

TO: Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
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In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been
filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☒ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED 5/8/2019	U.S. DISTRICT COURT for the District of Delaware
PLAINTIFF 10x Genomics, Inc.		DEFENDANT Celsee, Inc.
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1 10,155,981 B2	12/18/2018	10X Genomics, Inc.
2 10,227,648 B2	3/12/2019	10X Genomics, Inc.
3 10,240,197 B1	3/26/2019	10X Genomics, Inc.
4 10,273,541 B2	4/30/2019	10X Genomics, Inc.
5 10,280,459 B1	5/7/2019	10X Genomics, Inc.

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading		
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK	
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In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
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PARSE EX.1005.168

AO 120 (Rev. 08/16)

TO: Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
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In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court _____ for the District of Delaware _____ on the following

☐ Trademarks or ☒ Patents. (☐ the patent action involves 35 U.S.C. § 292.);

DOCKET NO. 18cv 1800- RGA	DATE FILED 11/15/2018	U.S. DISTRICT COURT for the District of Delaware
PLAINTIFF BECTON, DICKINSON AND COMPANY and CELLULAR RESEARCH, INC.		DEFENDANT 10X GENOMICS, INC.
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1 8,835,358 B2	9/16/2014	Becton, Dickinson and Company
2 9,315,857 B2	4/19/2016	Becton, Dickinson and Company
3 9,816,137 B2	11/14/2017	Becton, Dickinson and Company
4 9,290,809 B2	3/22/2016	Becton, Dickinson and Company
5 9,290,808 B2	3/22/2016	Becton, Dickinson and Company

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
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In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT <i>see attached Order</i>

CLERK <i>John A. Cerino</i>	(BY) DEPUTY CLERK <i>n. Selmya</i>	DATE <i>10/21/2019</i>
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Copy 2—Upon filing document adding patent(s), mail this copy to Director Copy 4—Case file copy

ADDENDUM TO AO 120 (ADDITIONAL PATENTS)

DOCKET NO.		DATE FILED 11/15/2018	U.S. DISTRICT COURT for the District of Delaware
PLAINTIFF BECTON, DICKINSON AND COMPANY and CELLULAR RESEARCH, INC.			DEFENDANT 10X GENOMICS, INC.
PATENT OR TRADEMARK NO.		DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
6	9,708,659 B2	7/18/2017	Becton, Dickinson and Company
7	9,845,502 B2	12/19/2017	Becton, Dickinson and Company
8	9,567,645 B2	2/14/2017	Becton, Dickinson and Company
9	9,567,646 B2	2/14/2017	Becton, Dickinson and Company
10	9,598,736 B2	3/21/2017	Becton, Dickinson and Company
11	9,637,799 B2	5/2/2017	Becton, Dickinson and Company

In the above---entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED		INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading			
PATENT OR TRADEMARK NO.		DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK		
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AO 120 (Rev. 08/10)

TO: Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
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In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court _____ for the District of Delaware _____ on the following

☐ Trademarks or ☒ Patents. (☐ the patent action involves 35 U.S.C. § 292.);

DOCKET NO. 18-1800-RGA	DATE FILED 11/15/2018	U.S. DISTRICT COURT for the District of Delaware
PLAINTIFF BECTON, DICKINSON AND COMPANY and CELLULAR RESEARCH, INC.		DEFENDANT 10X GENOMICS, INC.
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☒ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1 10,273,541 B2	4/30/2019	10X Genomics, Inc.
2 10,240,197 B1	3/26/2019	10X Genomics, Inc.
3 10,392,662 B1	8/27/2019	10X Genomics, Inc.
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In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
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AO 120 (Rev. 08/10)

TO: Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
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 filed in the U.S. District Court **for the District of Delaware** on the following

☐ Trademarks or ☒ Patents. (☐ the patent action involves 35 U.S.C. § 292.);

DOCKET NO. 18-1800-RGA	DATE FILED 11/15/2018	U.S. DISTRICT COURT for the District of Delaware
PLAINTIFF BECTON, DICKINSON AND COMPANY and CELLULAR RESEARCH, INC.		DEFENDANT 10X GENOMICS, INC.
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
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In the above entitled case, the following patent(s)/trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☒ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1 10,273,541 B2	4/30/2019	10X Genomics, Inc.
2 10,240,197 B1	3/26/2019	10X Genomics, Inc.
3 10,392,662 B1	8/27/2019	10X Genomics, Inc.
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In the above entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
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AO 120 (Rev. 08/10)

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☐ Trademarks or ☒ Patents. (☐ the patent action involves 35 U.S.C. § 292.);

DOCKET NO. 19-862-CFC-SRF		DATE FILED 5/8/2019	U.S. DISTRICT COURT for the District of Delaware
PLAINTIFF 10x Genomics, Inc.		DEFENDANT Celsee, Inc.	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK	
1 10,155,981 B2	12/18/2018	10X Genomics, Inc.	
2 10,227,648 B2	3/12/2019	10X Genomics, Inc.	
3 10,240,197 B1	3/26/2019	10X Genomics, Inc.	
4 10,273,541 B2	4/30/2019	10X Genomics, Inc.	
5 10,280,459 B1	5/7/2019	10X Genomics, Inc.	

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading		
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK	
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In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT Chief Judge Connolly signed the Stipulation of Dismissal on 7/27/2021.

CLERK John A. Cerin	(BY) DEPUTY CLERK [Signature]	DATE 7/28/2021
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AO 120 (Rev. 08/10)

TO: Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
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In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court _____ for the District of Delaware _____ on the following

☐ Trademarks or ☒ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO. 19-862-CFC-SRF	DATE FILED 5/8/2019	U.S. DISTRICT COURT for the District of Delaware
PLAINTIFF 10x Genomics, Inc.		DEFENDANT Celsee, Inc.
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY	
	☒ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1 10,392,662 B1	8/27/2019	10x Genomics, Inc.
2 10,400,280 B2	9/3/2019	10x Genomics, Inc.
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In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
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United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE	REQUEST ID
16/194,047	11/16/2018	Sydney Brenner	104371-5016-US10	165741

Acknowledgement of Loss of Entitlement to Entity Status Discount

The entity status change request below filed through Private PAIR on 05/10/2022 has been accepted.

CERTIFICATIONS:

Change of Entity Status:

☒ Applicant changing to regular undiscounted fee status.

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

This portion must be completed by the signatory or signatories making the entity status change in accordance with 37 CFR 1.4(d)(4).

Signature:	/Jeffrey Bolland/
Name:	Jeffrey Bolland
Registration Number:	73759

ADDENDUM TO AO 120 (ADDITIONAL PATENTS)

DOCKET NO.		DATE FILED 8/24/2022	U.S. DISTRICT COURT for the District of Delaware
PLAINTIFF 10X GENOMICS, INC.		DEFENDANT PARSE BIOSCIENCES, INC. and THE BOARD OF TRUSTEES OF LELAND STANFORD JUNIOR UNIVERSITY	
PATENT OR TRADEMARK NO.		DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
6	10,738,357	August 11, 2020	The Board of Trustees of the Leland Stanford Junior University
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED		INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading			
PATENT OR TRADEMARK NO.		DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK		
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