FORM 2

THE PATENTS ACT, 1970
(39 of 1970)
AND
THE PATENTS RULES, 2003

COMPLETE SPECIFICATION

(See Section 10; rule 13)

TITLE OF THE INVENTION

"POLYMERASE COMPOSITIONS, METHODS OF MAKING AND USING SAME"

APPLICANT

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The following specification particularly describes
the invention and the manner in which
it is to be performed

CLAIMS

1. A method for performing a nucleotide polymerization reaction, comprising:

contacting a modified polymerase or a biologically active fragment thereof in the presence of a high ionic strength solution with a nucleic acid template in the presence of one or more nucleotides, wherein the modified polymerase or the biologically active fragment thereof includes one or more amino acid modifications relative to a reference polymerase and has an increased dissociation time constant relative to the reference polymerase; and

polymerizing at least one of the one or more nucleotides using the modified polymerase or the biologically active fragment thereof.

2. The method of claim 1, wherein the polymerizing further includes polymerizing the at least one nucleotide in a template-dependent fashion.

3. The method of claim 1, further including hybridizing a primer to the nucleic acid template prior to, during or after the contacting, and wherein the polymerizing includes polymerizing the at least one nucleotide onto an end of the primer using the modified polymerase or the biologically active fragment thereof.

4. The method of claim 1, wherein the polymerizing is performed in the proximity of a sensor that is capable of detecting the polymerization of the at least one nucleotide by the modified polymerase or the biologically active fragment thereof.

5. The method of claim 1, further including detecting a signal indicating the polymerization of the at least one of the one or more nucleotides by the modified polymerase or the biologically active fragment thereof using a sensor.

6. The method of claim 5, wherein the sensor is an ISFET.

7. The method of claim 1, wherein the high ionic strength solution comprises about 200 mM to about 300 mM salt.

8. The method of claim 7, wherein the salt comprises NaCl and/or KCl.

9. The method of claim 1, wherein the modified polymerase or the biologically active fragment thereof comprises at least 100 amino acid residues having at least 90% identity to SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 19, SEQ ID NO: 20 or SEQ ID NO: 24.

10. A method for performing nucleic acid amplification, comprising:

generating an amplification reaction mixture having a modified polymerase or a biologically active fragment thereof, a primer, a nucleic acid template, and one or more nucleotides, wherein the modified polymerase or the biologically active fragment thereof includes one or more amino acid modifications relative to a reference polymerase and has an increased dissociation time constant relative to the reference polymerase; and

subjecting the amplification reaction mixture to amplifying conditions, wherein at least one of the one or more nucleotides is polymerized onto the end of the primer using the modified polymerase or the biologically active fragment thereof, and wherein the amplifying conditions include the presence of a high ionic strength solution.

11. The method of claim 10, wherein the high ionic strength solution comprises about 200 mM to about 300 mM NaCl or KCl.

12. The method of claim 10, wherein the amplifying conditions include amplification of the nucleic acid template by polymerase chain reaction, emulsion polymerase chain reaction, isothermal amplification, recombinase polymerase amplification or strand displacement amplification.

13. A method of detecting nucleotide incorporation, comprising:

performing a nucleotide incorporation using a modified polymerase or a biologically active fragment thereof in the presence of a high ionic strength solution; a nucleic acid template

and one or more nucleotide triphosphates; thereby generating one or more byproducts of the nucleotide incorporation; and

detecting the presence of at least one of the one or more byproducts of the nucleotide incorporation, thereby detecting the nucleotide incorporation.

14. The method of claim 13, wherein the detecting includes determining the identity of the nucleotide incorporation.

15. The method of claim 13, wherein the byproduct of the nucleotide incorporation is a hydrogen ion.

16. The method of claim 15, wherein the ion is a hydrogen ion.

17. A method for performing nucleic acid sequencing, comprising:

contacting a modified polymerase or a biologically active fragment thereof in the presence of a high ionic strength solution with a nucleic acid template in the presence of one or more nucleotides, wherein the modified polymerase or the biologically active fragment thereof includes one or more amino acid modifications relative to a reference polymerase, and wherein the modified polymerase or the biologically active fragment thereof has an increased dissociation time constant relative to the reference polymerase; and

polymerizing at least one of the one or more nucleotides using the modified polymerase or the biologically active fragment thereof.

18. The method of claim 17, wherein the one or more nucleotides do not contain a detectable label.

19. The method of claim 17, wherein the modified polymerase comprises at least 95% identity to SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO 24.

20. The method of claim 17, wherein the method further includes determining the identity of the one or more nucleotides polymerized by the modified polymerase.

Dated this 10 day of March 2014

(Arindam Paul)
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Of De Penning & De Penning
Agent for the Applicants

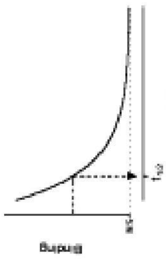
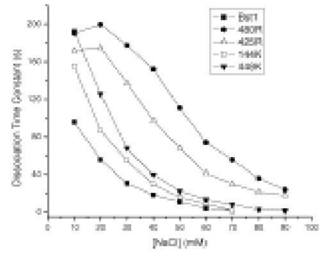


FIG. 1

FIG. 2



Dissociation Time Constant (s)
[NaCl] (mM)
0
40
80
120
160
200
10
20
30
40
50
60
70
80
90
100

FIG. 3

Amino Acid	Number of hits at this Amino Acid Position	Protein Activity	Relative template affinity vs. WT
[illegible]		[illegible]	[illegible]
[illegible]	15	[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]	5	[illegible]	[illegible]
[illegible]	4	[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]	4	[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]	4	[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]	4	[illegible]	[illegible]
[illegible]		[illegible]	[illegible]
[illegible]		[illegible]	[illegible]

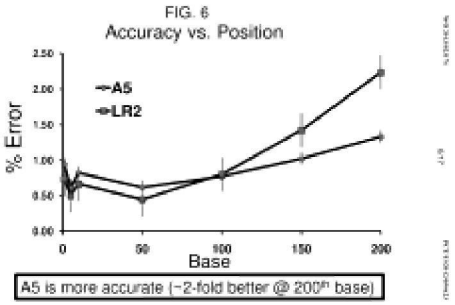
FIG. 4

FIG. 5

polymerase	noise ratio	% above 5% error	CCTT del rate	TA_TAGGC del rate
N1	7.05	0.62	0.68	1.87
N3	7.13	0.62	0.74	1.85
N5	6.85	0.62	0.67	1.76
N7	6.85	0.67	0.63	1.88
AVG	6.971	0.63	0.68	1.73
LR2	6.71	1.08		7.48
LR2	6.93	1.01	0.63	7.04
LR2	6.65	0.96	0.64	8.16
LR2	6.71	1.06		7.67
LR2	6.65	1.10	0.59	9.23
LR2	6.71	1.10	0.66	8.19
AVG	6.68	1.06	0.63	8.01
T Test LR2 v N5	0%	0%	91%	0%

A5 significantly decreases error at certain high deletion rate motifs

FIG. 6


Accuracy vs. Position
% Error
Base
A5
LR2
2.50
2.00
1.50
1.00
0.50
0.00
0
50
100
150
200
A5 is more accurate (~2-fold better @ 200th base)

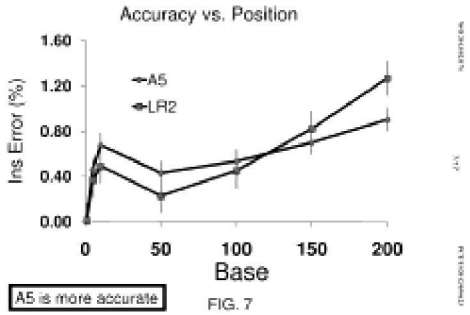
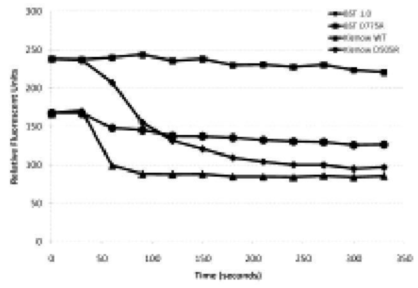

Accuracy vs. Position
1.60
1.20
0.80
0.40
0.00
Ins Error (%)
A5
LR2
0
50
100
150
200
Base
A5 is more accurate

FIG. 7

FIG. 8


BST 1.0
BST D715R
Klenow WT
Klenow D505R
Relative Fluorescent Units
Time (seconds)
0
50
100
150
200
250
300
350

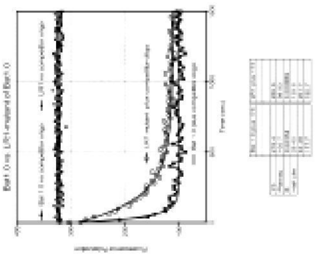


FIG. 9

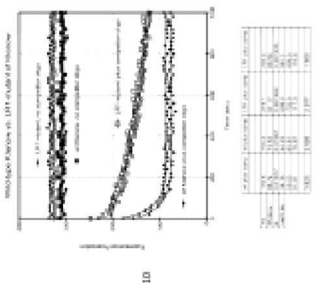
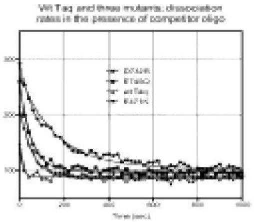


FIG. 10

FIG. 11

FIG. 12



	[illegible]	[illegible]	wt Taq	[illegible]
Best-fit values				
Y0	[illegible]	[illegible]	[illegible]	[illegible]
NS	[illegible]	[illegible]	[illegible]	[illegible]
K	[illegible]	[illegible]	[illegible]	[illegible]
Half-life	[illegible]	[illegible]	[illegible]	[illegible]

FIG. 13

run name	aq17 bases	aq20 bases	aq17 mean	aq20 mean	library reads	aq7 alignments	200q17 / aq7 alignments
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AVERAGE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

FIG. 16

FIG. 15

Filtered Q17 Coverage Percentage:	100.00
Filtered Q17 Mean Coverage Depth:	220.8
Filtered Q17 Alignments:	2,923,063
Filtered Q17 Mean Alignment Length:	350
Filtered Mapped Bases in Q17 Alignments:	1,024,257,977
Filtered Q17 Longest Alignment:	482 base pairs
Filtered Q20 Coverage Percentage:	100.00
Filtered Q20 Mean Coverage Depth:	191.8
Filtered Q20 Alignments:	2,858,185
Filtered Q20 Mean Alignment Length:	311
Filtered Mapped Bases in Q20 Alignments:	890,267,209
Filtered Q20 Longest Alignment:	471 base pairs

FIG. 16

Filtered Q17 Coverage Percentage:	100.00
Filtered Q17 Mean Coverage Depth:	221.0
Filtered Q17 Alignments:	3,358,199
Filtered Q17 Mean Alignment Length:	306
Filtered Mapped Bases in Q17 Alignments:	1,025,247,955
Filtered Q17 Longest Alignment:	443 base pairs
Filtered Q20 Coverage Percentage:	100.00
Filtered Q20 Mean Coverage Depth:	196.0
Filtered Q20 Alignments:	3,267,129
Filtered Q20 Mean Alignment Length:	277
Filtered Mapped Bases in Q20 Alignments:	904,914,822
Filtered Q20 Longest Alignment:	443 base pairs

FIG. 17

Pol	Ag17 Bases	Ag20 Bases	Key Signal	Ag17 mean	S/N	No of Reads
Taq	361,845,128	296,166,931	41	152	9	3,181,990
Taq	139,445,171	108,093,671	45	136	8	1,871,475
Taq	388,598,112	331,215,085	45	180	9	2,489,655
Taq	151,478,898	121,089,170	45	131	8	1,837,918
AVG	263,341,957	211,640,564	44	148	9	2,346,275
LR1/Taq	1,147,086,398	1,002,171,814	51	331	12	3,585,527
LR1/Taq	759,445,416	634,727,054	48	271	10	3,081,475
LR1/Taq	688,911,564	590,171,449	50	255	10	3,030,389
LR1/Taq	901,449,079	788,358,426	51	335	13	3,081,835
AVG	874,218,058	753,908,676	50	281	11	3,170,206
T-Test	0%	8%	0%	0%	3%	2%

POLYMERASE COMPOSITIONS, METHODS OF MAKING AND USING SAME

[0001] CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 61/522,125, filed August 10, 2011, U.S. Provisional Application No.

61/545,434, filed October 10, 2011, U.S. Provisional Application No. 61/585,133, filed January 10, 2012, U.S. Provisional Application No. 61/643,844 filed May 7, 2012, and U.S. Provisional Application No. 61/681,593 filed August 9, 2012 entitled "POLYMERASE COMPOSITIONS, METHODS OF MAKING AND USING THE SAME", the disclosures of which are incorporated herein by reference in their entireties.

[0003] SEQUENCE LISTING

[0004] This application hereby incorporates by reference the material of the electronic Sequence Listing filed concurrently herewith. The material in the electronic Sequence Listing is submitted as a text (.txt) file entitled "LT00556PCT _Sequence_Listing_ST25.txt" created on August 9, 2012, which has a file size of 65 KB, and is herein incorporated by reference in its entirety.

[0005] TECHNICAL FIELD

[0006] In some embodiments, the disclosure relates generally to polymerase compositions,

methods of making and using the same. In some embodiments, the disclosure relates generally to one or more modified polymerases, where the one or more modified polymerases contain at least one amino acid mutation as compared to a reference polymerase. In some embodiments, the disclosure relates generally to compositions comprising a modified DNA or RNA polymerase. In some embodiments, the compositions can include a modified polymerase from an A family DNA polymerase or a B family DNA polymerase. In some embodiments, the disclosure relates generally to the transfer of a homologous amino acid mutation across a class or family of polymerases. In some embodiments, the disclosure relates generally to a polymerase composition for nucleic acid sequencing, including next- generation sequencing. In some embodiments, the disclosure relates generally to a modified polymerase composition for the generation of nucleic acid libraries or nucleic acid templates. In some embodiments, the disclosure relates to systems, apparatuses and kits that contain one or more of the modified polymerases. In some embodiments, the compositions, systems, apparatuses and kits can be used for synthesizing a DNA strand. In some embodiments, the compositions, systems,

apparatuses and kits can be used for amplifying at least 10, 50, 100, 500, 1000, 2500, 5000, 7500, 10000, 25000, 50000, 100000, or more nucleic acid templates in a single reaction.

[0007] BACKGROUND

[0008] The ability of enzymes to catalyze biological reactions is fundamental to life. A range of biological applications use enzymes to synthesize various biomolecules in vitro. One particularly useful class of enzymes is the polymerases, which can catalyze the polymerization of biomolecules (e.g., nucleotides or amino acids) into biopolymers (e.g., nucleic acids or peptides). For example, polymerases that can polymerize nucleotides into nucleic acids, particularly in a template-dependent fashion, are useful in recombinant DNA technology and nucleic acid sequencing applications. Many nucleic acid sequencing methods monitor nucleotide incorporations during in vitro template-dependent nucleic acid synthesis catalyzed by a polymerase. Single Molecule Sequencing (SMS) and Paired-End Sequencing (PES) typically include a polymerase for template-dependent nucleic acid synthesis. Polymerases are also useful for the generation of nucleic acid libraries, such as libraries created during emulsion PCR or bridge PCR. Nucleic acid libraries created using such polymerases can be used in a variety of downstream processes, such as genotyping, nucleotide polymorphism (SNP) analysis, copy number variation analysis, epigenetic analysis, gene expression analysis, hybridization arrays, analysis of gene mutations including but not limited to detection, prognosis and/or diagnosis of disease states, detection and analysis of rare or low frequency allele mutations, and nucleic acid sequencing including but not limited to de novo sequencing or targeted resequencing.

[0009] When performing polymerase-dependent nucleic acid synthesis or amplification, it can be useful to modify the polymerase (for example via mutation or chemical modification) so as to alter its catalytic properties. In some instances, it can be useful to modify the polymerase to enhance its catalytic properties. Polymerase performance in various biological assays involving nucleic acid synthesis can be limited by the kinetic behavior of the polymerase towards nucleotide substrates. For example, analysis of polymerase activity can be complicated by undesirable behavior such as the tendency of a given polymerase to dissociate from the template; to bind and/or incorporate the incorrect, e.g., non Watson-Crick base- paired, nucleotide; or to release the correct, e.g., Watson-Crick based paired, nucleotide without incorporation. These and other desirable properties can be enhanced via suitable selection, engineering and/or modification of a polymerase of choice. For example, such

modification can be performed to favorably alter the polymerase's rate of nucleotide incorporation, affinity of binding to template, processivity or average read length; such alterations can increase the amount of sequence information obtained from a single sequencing reaction. There remains a need in the art for improved polymerase compositions exhibiting altered, e.g., increased processivity, read length (including error-free read length) and/or affinity for DNA template. Such polymerase compositions can be useful in a wide variety of assays involving polymerase-dependent nucleic acid synthesis, including nucleic acid sequencing and production of nucleic acid libraries.

[0010] BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The accompanying drawings, which are incorporated into and form a part of the specification, illustrate one or more exemplary embodiments and serve to explain the principles of various exemplary embodiments. The drawings are exemplary and explanatory only and are not to be construed as limiting or