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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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(88) **Date of publication of the international search report**:
5 January 2017

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(54) **Title**: POLYMERASE VARIANTS AND USES THEREOF

(57) **Abstract**: Described herein is a variant pol6 polymerase having at least one mutation selected from H223, N224, Y225, H227, I295, Y342, T343, I357, S360, L361, I363, S365, S366, Y367, P368, D417, E475, Y476, F478, K518, H527, T529, M531, N535, G539, P542, N545, Q546, A547, L549, I550, N552, G553, F558, A596, G603, A610, V615, Y622, C623, D624, I628, Y629, R632, N635, M641, A643, I644, T647, I648, T651, I652, K655, W656, D657, V658, H660, F662, L690 and combinations thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/32258

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 1/00, C07K 14/00, C07K 17/00, C12N 9/12 (2016.01)
CPC - A61K 38/00, C07K 14/47, C07K 2319/00, C07K 14/705, A61K 39/00, C12N 9/00, C12N 9/1252
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07K 1/00, C07K 14/00, C07K 17/00, C12N 9/12 (2016.01)
CPC - A61K 38/00, C07K 14/47, C07K 2319/00, C07K 14/705, A61K 39/00, C12N 9/00, C12N 9/1252

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC- C12Y 207/07007
USPC- 530/350, 435/194, 435/6.12, 435/91.2, 435/91.5

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: DNA polymerase, Clostridium perfringens, 3' 5' exonuclease activity, DNA_pol_B, Clostridium phage phiZP2, dsDNA viruses, Clostridium phage phiCPV4, Type B polymerase, I3PV37_9CAUD, GenBank NCBI JQ729991 [phi]CPV4... GenCore 6.4.1: SEQ ID NO:2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Volozhantsev, et al. Molecular Characterization of Podoviral Bacteriophages Virulent for Clostridium perfringens and Their Comparison with Members of the Picovirinae. PLoS ONE 2012, 7:e38283-e38283; pg 3 col 1; pg 10, col 2;	1-6, 19, 23
A	UniProt Submission I3PV37_9CAUD (01 April 2015) [Retrieved from the Internet 18 August 2016: <http://www.uniprot.org/uniprot/I3PV37.txt?version=12>]; 100% identity to SEQ ID NO:1	1-6, 19, 23
A	Kranaster, et al. Taking fingerprints of DNA polymerases: multiplex enzyme profiling on DNA arrays. Angew Chem Int Ed Engl. 2009, 48(25):4625-4628; pg 4625, col 1; pg 4627	1-6, 19, 23

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 August 2016	28 NOV 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/32258

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group I+: claims 1-23, directed to a modified DNA polymerase having a DNA polymerase activity comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 1 or 2. The modified DNA polymerase will be searched to the extent that it encompasses H223 mutation [NOTE: numeration is according to SEQ ID NO:2]. It is believed that claims 1-6, 19, 23 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a modified DNA polymerase having at least 70% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 1 or 2. The modified DNA polymerase and having a H223 mutation. An additional mutation(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected mutation(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be an S366 mutation, I.e., claims 1-7, 19-23.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 19, 23, restricted to a H223 mutation

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

********** Supplemental Sheet **********

In Continuation of Box III. Observations where unity of invention is lacking:

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
The special technical feature of each invention of Group I+ is a specific mutation recited therein, because a nucleic sequence having at least 70% identity with SEQ ID NO: 1 or SEQ ID NO:2 was known in the art at the time of the invention (please see UniProt Submission I3PV37_9CAUD (01 April 2015) [Retrieved from the Internet 18 August 2016: <http://www.uniprot.org/uniprot/I3PV37.txt?version=12>] (hereinafter "UniProt") disclosing DNA polymerase of Clostridium phage phiCPV4, wherein amino acids 2-727 displays 100% identity to amino acids 2-727 of SEQ ID NO: 1 and amino acids 14-739 of SEQ ID NO: 2), and, therefore, no significant structural similarities can readily be ascertained among the mutations.

Common Technical Features
The inventions of Group I+ share the technical feature of a modified DNA polymerase having a DNA polymerase activity and at least 70% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 1 or 2. However, this shared technical feature does not represent a contribution over prior art as being obvious over a paper titled "Molecular Characterization of Podoviral Bacteriophages Virulent for Clostridium perfringens and Their Comparison with Members of the Picovirinae" by Volozhantsev, et al. (PLoS ONE 2012, 7:e38283-e38283) (hereinafter "Volozhantsev") in view of a paper titled "Taking fingerprints of DNA polymerases: multiplex enzyme profiling on DNA arrays" by Kranaster, et al. (Angew Chem Int Ed Engl. 2009, 48(25):4625-8) (hereinafter "Kranaster") as follows:

Volozhantsev discloses a predicted DNA polymerase (pg 3, col 1, "The predicted Type B polymerases of all three phages were 727 amino acids in length and [phi]CPV4 and [phi]CP7R were more closely related (99% sequence similarity) than either one compared to the [phi]ZP2 (96% similar to [phi]CPV4 and [phi]CP7R). BLAST analyses revealed the three phage proteins were most closely related in sequence to the [phi]CPV1 [GenBank ADR30478; 36%] and [phi]CP24R [AEW47837; 34%] polymerases with overall similarity of 24% to other DNA polymerases of the Bacillus [phi]29-family") comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 1 or 2 (pg 10, col 2, "GenBank NCBI accession numbers for the genomes were assigned as ... JQ729991 for [phi]CPV4", said genome comprising a nucleic acid encoding an amino acid sequence having at least 70% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 1 or 2 (see UniProt, as above, "DR EMBL; JQ729991... Genomic_DNA"; amino acids 2-727, 100% identity to amino acids 2-727 of SEQ ID NO: 1 and amino acids 14-739 of SEQ ID NO: 2). Volozhantsev neither discloses that said DNA polymerase is modified nor provides a motivation to do so.
The motivation is provided by Kranaster disclosing a need for improved polymerases exhibiting altered properties (pg 4625, col 1, "customized and artificially engineered DNA polymerases that lead to more robust andspecific reaction systems are urgently needed") and further disclosing a "microarray-based approach for DNA polymerase evolution which we term oligonucleotide-addressing enzyme assay (OAEA). First studies have proven the practicability of our approach and identified new DNA polymerase mutants with altered properties. In comparison with other known directed evolution approaches for DNA polymerases, OAEA offers severalsignificant advantages. First, this approach allows the multiplex detection of various DNA polymerase activities in parallel under identical conditions. In addition, in OAEA each reaction can be duplicated readily. These features render OAEA reliable and less prone to false positives and negatives. Furthermore, all steps can be performed by automated pipetting devices, allowing high-throughput analysis requiring only minuscule amounts of reagents" (pg 4627, col 1 to col 2). It would have been obvious to one of ordinary skill in the art to combine, in the course of routine experimentation and with a reasonable expectation of success, Volozhantsev with Kranaster by applying the screening method of Kranaster to a library of mutagenized [phi]CPV4 DNA polymerase disclosed by, to achieve a customized DNA polymerase. As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the inventions.

The inventions of Group I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.