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(54) Title: RECOMBINANT REVERSE TRANSCRIPTASE VARIANTS FOR IMPROVED PERFORMANCE

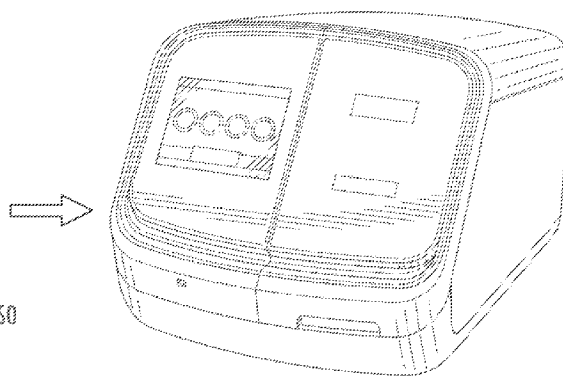
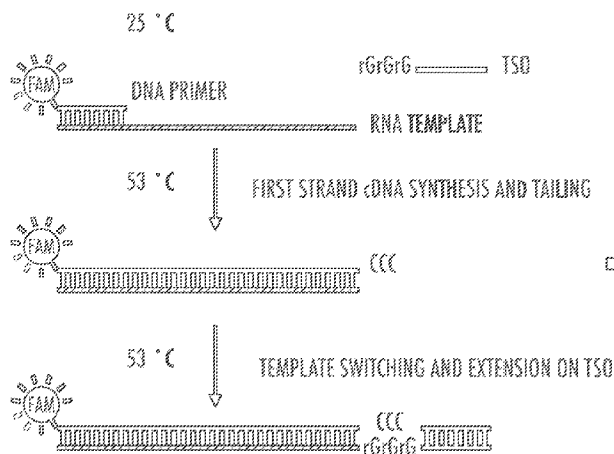


FIG. 1

(57) Abstract: The disclosure provides recombinant reverse transcriptases comprising one or more DNA binding domains conjugated to an engineered reverse transcriptase that have been modified to exhibit one or more altered reverse transcriptase related activities such as but not limited to altered template switching efficiency, altered transcription efficiency or both. The disclosure further provides compositions and kits comprising the recombinant reverse transcriptase enzymes and methods of producing, amplifying or sequencing nucleic acid molecules using these fusion reverse transcriptase enzymes.



RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS,
ZA, ZM, ZW.

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Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/12
ADD.

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)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/200867 A1 (10X GENOMICS INC [US]) 1 November 2018 (2018-11-01) sequences 5, 6 paragraph [0306]; example 8 -----	1, 3-5, 7, 17, 18, 47-50, 67, 68, 82-85
X	WO 2007/022045 A2 (STRATAGENE CALIFORNIA [US]; HOGREFE HOLLY [US] ET AL.) 22 February 2007 (2007-02-22) sequence 35 ----- ----- -/--	1, 3-5, 7, 17, 18, 47-50, 67, 68, 82-85

Further documents are listed in the continuation of Box C.

See patent family annex.

* 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

19 April 2023

23/06/2023

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INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 2020/191239 A1 (BROAD INST INC [US]; HARVARD COLLEGE [US]) 24 September 2020 (2020-09-24)</p> <p>the whole document paragraph [1291] sequences 740, 741</p> <p>-----</p>	<p>1, 3-5, 7, 17, 18, 47-50, 67, 68, 82-85</p>
X	<p>WO 2021/119320 A2 (10X GENOMICS INC [US]) 17 June 2021 (2021-06-17)</p> <p>-----</p>	<p>1, 3, 7, 17, 18, 47-50, 67, 68, 82-85</p>
Y	<p>the whole document example 3 figure 6 sequence 1</p> <p>-----</p>	<p>20-25, 32-35, 37-46, 70-81</p>
X	<p>WO 2021/178720 A2 (FLAGSHIP PIONEERING INNOVATIONS VI LLC [US]) 10 September 2021 (2021-09-10) sequence 19</p> <p>-----</p>	<p>69</p>
X	<p>CN 112 442 493 A (GUANGDONG FAPON BIOLOGICAL CO LTD) 5 March 2021 (2021-03-05)</p> <p>-----</p>	<p>12, 17, 18, 26-31, 36, 50-66</p>
Y	<p>example sequence 6</p> <p>-----</p>	<p>20-25, 32-35, 37-46, 70-81</p>
X,P	<p>WO 2022/232571 A1 (10X GENOMICS INC [US]) 3 November 2022 (2022-11-03)</p> <p>the whole document</p> <p>-----</p>	<p>1, 3-5, 7, 12, 17, 18, 20-85</p>

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/053174

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. forming part of the international application as filed.
 - b. furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)).
 accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
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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:

see additional sheet

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 an 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, 3-5, 7, 12, 17, 18, 20-85(all partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 3-5, 7, 12, 17, 18, 20-85(all partially)

The reverse transcriptase comprising the following combination of mutations departing from SEQ ID NO:7 or 178:(a) (a) E69K, L139P, E302R, T306K, W313F, T330P, N454K; and one or more of M39V, P47L, Q91R, M66L, F155Y, D200N, D200E, H204R, G429S, L435G, L435K, P448A, D449G, H503V, D524N, T542D, E545G, D583N, H594Q, L603W, L603F, E607K, E607G, P627S, H634Y, H638G, A644V, D653H, K658R and L671P; or (b) E69K, L139P, D200N, E302R, T306K, W313F, T330P, L435G, P448A, D449G, N454K, D524N, L603W, and E607K and one or more of M39V, P47L, M66L, Q91R, F155Y, H204R, G429S, H503V, T542D, E545G, D583N, H594Q, P627S, H634Y, H638G, A644V, D653H, K658R and L671P; wherein the RT comprises the amino acid sequence of SEQ ID NO:1; fusion reverse transcriptase comprising a DNA-binding domain thereof; nucleic acid encoding the same; expression vector, host cell, methods, compositions and kits thereof.

2. claims: 13-16(completely); 1-5, 7-12, 17-85(partially)

Idem to invention 1 but related to SEQ ID NO:143

- 3-30. claims: 1-12, 17-85(all partially)

invention 3 being idem to invention 1 but related to SEQ ID NO:180; invention 4 being idem to invention 1 but related to SEQ ID NO:181; [...] ; invention 30 being idem to invention 1 but related to SEQ ID NO:208.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/053174

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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