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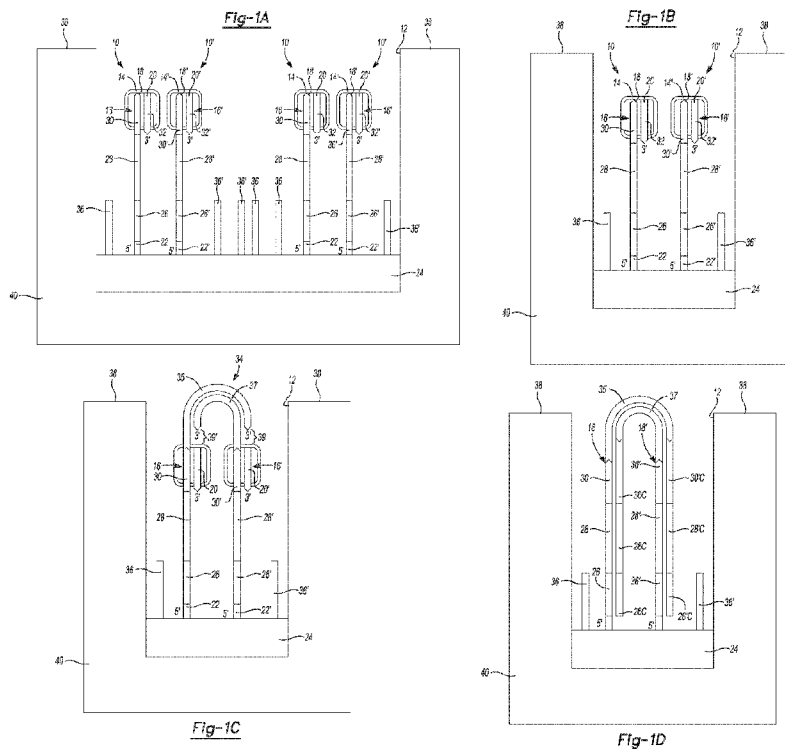
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(54) Title: FLOW CELLS AND METHODS



(57) Abstract: An example of a flow cell includes a substrate having depressions separated by interstitial regions. First and second primers are immobilized within the depressions. First transposome complexes are immobilized within the depressions, and the first transposome complexes include a first amplification domain. Second transposome complexes are also immobilized within the depressions, and the second transposome complexes include a second amplification domain. Some of the first transposome complexes, or some of the second transposome complexes, or some of both of the first and second transposome complexes include a modification to reduce tagmentation efficiency.



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- *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))*
- *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/082280

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>KLENCHIN VADIM A. ET AL: "Phosphate coordination and movement of DNA in the Tn5 synaptic complex: role of the (R)YREK motif", NUCLEIC ACIDS RESEARCH, vol. 36, no. 18, October 2008 (2008-10), pages 5855-5862, XP093040115, GB ISSN: 0305-1048, DOI: 10.1093/nar/gkn577 Retrieved from the Internet: URL:https://biochem.wisc.edu/sites/default/files/people/ivan-rayment/pub_pdfs/160_179/Ivan's_CV_177.pdf> abstract</p>	2
A	<p>VAEZESLAMI SOHEILA ET AL: "Site-Directed Mutagenesis Studies of Tn 5 Transposase Residues Involved in Synaptic Complex Formation", JOURNAL OF BACTERIOLOGY, vol. 189, no. 20, 15 October 2007 (2007-10-15), pages 7436-7441, XP093039909, US ISSN: 0021-9193, DOI: 10.1128/JB.00524-07 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2168436/pdf/0524-07.pdf> abstract page 7438, left-hand column, paragraph 2</p>	3, 4
A	<p>FULLER JAMES R ET AL: "Target DNA bending by the Mu transpososome promotes careful transposition and prevents its reversal", ELIFE, vol. 6, 13 February 2017 (2017-02-13), XP093040255, DOI: 10.7554/eLife.21777 Retrieved from the Internet: URL:https://cdn.elifesciences.org/articles/21777/elifesciences-21777-v3.xml> page 5, last paragraph Legend of Fig 3 - Fig suppl. 1; page 6</p>	5
A	<p>WO 2016/003814 A1 (ILLUMINA INC [US]) 7 January 2016 (2016-01-07) page 2, paragraph 3 page 7, paragraph 2 example 4</p>	5
9 1	<p>WO 2018/156519 A1 (ILLUMINA INC [US]; ILLUMINA CAMBRIDGE LTD [GB]) 30 August 2018 (2018-08-30) paragraph [0122]; figure 1</p>	1-5
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/082280

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>ADEY ANDREW C.: "Tagmentation-based single-cell genomics", GENOME RESEARCH, vol. 31, no. 10, October 2021 (2021-10), pages 1693-1705, XP093040149, US ISSN: 1088-9051, DOI: 10.1101/gr.275223.121 Retrieved from the Internet: URL: https://genome.cshlp.org/content/31/10/1693.full.pdf#page=1&view=FitH the whole document</p> <p style="text-align: center;">-----</p>	1-5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/082280

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.
PCT/US2022/082280

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.:
1-5

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: 1-5

A flow cell comprising transposome complexes with a modification to reduce tagmentation efficiency.

2. claims: 6, 7

A flow cell comprising first and second transposome complexes immobilised in different areas.

3. claims: 8, 9

A method for increasing an insert size of a deoxyribonucleic acid sample comprising the use of a condensation agent or a tagmentation inhibitor.

4. claims: 10-12

A method comprising attaching transposome complexes to hydrogel in flow cell depressions via alkyne functional groups.

5. claims: 13-20, 72-79

A method comprising attaching transposome complexes to hydrogel in flow cell depressions via biotin. Related reusable flow cell. A tagmentation kit comprising a flow cell and a biotin-containing linker.

6. claims: 21-36

A method comprising generating a complexed crude lysate comprising the use of a chaotropic detergent.

7. claims: 37-40

A method comprising performing a tagmentation reaction with a surface bound transposome complex comprising the use of a chaotropic detergent.

8. claims: 41-49

A flow cell comprising first and second asymmetrically attached transposome complexes present at a particular ratio. Related method.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

9. claims: 50-58

A flow cell comprising a first and second hydrogel in different portions of the depressions, immobilised transposome precursor and immobilised capture primer or transposome capture functional group. Related kit and tagmentation method.

10. claims: 59-71

A method comprising generating a crude lysate using zinc oxide nanomaterials and exposing it to tagmentation.

11. claims: 80-83

A flow cell comprising multi-depth depressions and a hydrogel. Related preparation method.

12. claims: 84-91

A method comprising tagmentation of DNA in two steps.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/082280

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