# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

# PCT

## WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
*(day/month/year)* see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

**FOR FURTHER ACTION**
See paragraph 2 below

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/EP2018/068441 | 06.07.2018 | 07.07.2017 |

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/6806 C12Q1/6883 C12Q1/6886

Applicant
NIPD GENETICS PUBLIC COMPANY LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA: | Date of completion of this opinion | Authorized Officer |
|---|---|---|
| European Patent Office<br>P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040<br>Fax: +31 70 340 - 3016 | see form PCT/ISA/210 | Gabriels, Jan<br>Telephone No. +31 70 340-0 |

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2018/068441

**Box No. I Basis of the opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-28 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-28 |
| Industrial applicability (IA) | Yes: | Claims | 1-28 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1.1 **CITATIONS**

Reference is made to the following documents:

D1 WO 2014/130890 A1 (TOMA BIOSCIENCES INC [US]) 28 August 2014 (2014-08-28)

D2 WO 2012/092426 A1 (FOUNDATION MEDICINE INC [US]; DOWNING SEAN R [US]; JAROSZ MIRNA [US];) 5 July 2012 (2012-07-05)

D3 WO 2016/189388 A1 (NIPD GENETICS LTD [CY]) 1 December 2016 (2016-12-01)

D4 ERIC J DUNCAVAGE ET AL: "Targeted next generation sequencing of clinically significant gene mutations and translocations in leukemia",
MODERN PATHOLOGY,
vol. 25, no. 6, 16 March 2012 (2012-03-16), pages 795-804, XP055235397,
GB
ISSN: 0893-3952, DOI: 10.1038/modpathol.2012.29

D5 EP 2 902 500 A1 (NATERA INC [US]) 5 August 2015 (2015-08-05)

D6 US 2017/051355 A1 (ZIMMERMANN BERNHARD [US] ET AL) 23 February 2017 (2017-02-23)

D7 US 2015/203907 A1 (GILBERT DAVID M [US] ET AL) 23 July 2015 (2015-07-23)

D8 US 2011/039304 A1 (CHURCH GEORGE M [US] ET AL) 17 February 2011 (2011-02-17)

D9 US 2008/194414 A1 (ALBERT THOMAS J [US] ET AL) 14 August 2008 (2008-08-14)

D10 WO 2016/024134 A2 (OXFORD GENE TECHNOLOGY OPERATIONS LTD [GB]) 18 February 2016 (2016-02-18)

D11 US 2016/068889 A1 (GOLE JEFF [US] ET AL) 10 March 2016 (2016-03-10)

D12 XI LIN ET AL: "Applications of targeted gene capture and next-generation sequencing technologies in studies of human deafness and other genetic disabilities", HEARING RESEARCH, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 288, no. 1, 6 January 2012 (2012-01-06), pages 67-76, XP028519246, ISSN: 0378-5955, DOI: 10.1016/J.HEARES.2012.01.004 [retrieved on 2012-01-14]

1.2 **NOVELTY** (Art. 33(2) PCT)

1.3 D1 discloses method of assessing cancer, comprising: (a) determining a presence, absence, and/or amount of each of a subset of genes in a sample derived from a fluid sample in a subject, wherein the subset is determined by (i) performing targeted sequencing on a set of genes on a solid tissue sample from the subject wherein the solid tissue sample is known or suspected of comprising cancerous tissue; (ii) determining a profile of genetic abnormalities for said set of genes based on the targeted sequencing; and (iii) selecting a subset of genes of the set of genes based on said profile for said set, wherein said subset is specific to said subject; and (b) from the results of step (a) determining the status of the cancer in the subject. D1 also uses tiling (cf. paragraph 540).

1.4 D2 discloses a method of analyzing a tumor sample, comprising: (a) acquiring a library comprising a plurality of tumor members from a tumor sample; (b) contacting the library with a plurality of bait sets to provide selected members, thereby providing a library catch;(c) acquiring a read for a subgenomic interval from a tumor member from said library or library catch; (d) aligning said read; and (e) assigning a nucleotide value from said read for a preselected nucleotide position for a preselected nucleotide position in each of a plurality

of subgenomic intervals, thereby analyzing said tumor sample. D2 uses a staggered set of bait molecules target a region 8(cf. page 95 claims 1-11, example 13).

1.4.1 D3 discloses a method of testing for risk of a chromosomal abnormality in fetal DNA in a mixed sample of maternal and fetal DNA, the method comprising: (a) preparing a sequencing library from the mixed sample; (b) hybridizing the sequencing library to a pool of TArget Capture Sequences (TACS), wherein the pool of TACS comprises sequences that bind to one or more chromosomes of interest comprising a chromosomal abnormality and wherein: (i) each sequence within the pool is between 100-260 base pairs in length, each sequence having a 5' end and a 3' end; (ii) each sequence within the pool binds to the chromosome(s) of interest at least 150 base pairs away, on both the 5' end and the 3' end, from regions harboring Copy Number Variations (CNVs), Segmental duplications or repetitive DNA elements; and (ii) the GC content of the TACS is between 19% and 50%; (c) isolating members of the sequencing library that bind to the TACS to obtain an enriched library; (d) amplifying and sequencing the enriched library; and (e) performing statistical analysis on the enriched library sequences to thereby determine a risk of the chromosomal abnormality in the fetal DNA (cf. claims 1, 5-22). D3 does not disclose detecting tumor biomarkers.

1.4.2 D4 discloses the use of capture probes for targeted next generation sequencing for identifying clinically significant gene mutations and translocations in leukemia. D2 uses Agilent SureSelect probes designed to 2 x tile across genes of interest in leukemia biology and prognosis (Table 1).

1.4.3 D5 discloses targeted capture based disease screening tests and discusses probe design including overlapping and tiled probes in paragraphs 150-153.

1.4.4 D6 discloses similar subject-matter as D3 (cf. paragraphs 303-306). D4 further relates to a method for determining a ploidy status of a chromosome or chromosome segment of a cancer in a host (cf. claim 1).

1.4.5 D7 discloses in figure 2 and paragraph 129 a plurality of capture oligos 260, or capture probes, designed to be able to hybridize to their complementary DNA sequences within the plurality of capture regions 180 in genomic DNA 110, so the plurality of capture oligos 260 is able to capture the plurality of capture regions 180 from a genomic DNA. To achieve a high enrichment outcome, the plurality of capture oligos is preferred to overlap each other and tile the plurality of capture regions.

1.4.6 D8 discloses the use of tiling in a method for enriching a target nucleic acid sequence (cf. example 1).

1.4.7 D9 discloses a method of reducing the genetic complexity of a population of nucleic acid molecules using a microarray. The probes of this array may be either designed to be overlapping probes, meaning that the starting nucleotides of adjacent probes are separated in the genome by less than the length of a probe, or non-overlapping probes, where the distance between adjacent probes are greater than the length of a probe. The distance between adjacent probes is generally overlapping, with spacing between the starting nucleotide of two probes varying between 1 and 100 bases (cf. paragraph 62).

1.4.8 D10 discloses the use of overlapping probe sets on microbeads for enriching a sample for a nucleic acid of interest (cf. page 5).

1.4.9 D11 discloses in paragraph 57 that s set of probes for a given target can be designed to 'tile' across the target, capturing the target as a series of shorter sub targets. In some embodiments, where a set of probes for a given target is designed to 'tile' across the target, some probes in the set capture flanking non-target sequence). Alternately, the set can be designed to 'stagger' the exact positions of the hybridization regions flanking the target, capturing the full target (and in some cases capturing flanking non-target sequence) with multiple probes having different targeting arms, obviating the need for tiling. The particular approach chosen will depend on the nature of the target set. For example, if small regions are to be captured, a staggered-end approach might be appropriate, whereas if longer regions are desired, tiling might be chosen.

1.4.10 D12 discloses that in targeted enrichment, a tiling scheme which allows overlapped probes to target the region of interest is generally used to increase capture efficiency (cf. page 69, left-hand column).

1.4.11 The present application does satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of claims 1-28 is new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

1.5 **INVENTIVE STEP** (Art. 33(3) PCT)

1.5.1 Document D1 is considered to represent the most relevant state of the art for claim 1. The subject-matter of claim 1 differs in that TACS are used in the method.

1.5.2 The problem to be solved by the subject matter of claim 1 may therefore be regarded as providing an alternative capture probe design.

1.5.3 This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

1.5.4 TACS were already known from D3. The skilled person would use them in these methods.

1.5.5 Document D1 is also considered to represent the most relevant state of the art for claim 2. The subject-matter of claim 2 differs in that each TACS family member sequence binds to the same genomic sequence of interest but has different start and/or stop positions with respect to a reference coordinate system for the genomic sequence of interest.

1.5.6 The problem to be solved by the subject matter of claim 2 may therefore be regarded as providing an improved enrichment method using TACS. The solution would be that each TACS family member sequence binds to the same genomic sequence of interest but has different start and/or stop positions with respect to a reference coordinate system for the genomic sequence of interest.

1.5.7 This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

1.5.7.1 The use of probes with staggered or overlapping start and stop positions was already known from the prior art as shown in any of D4 to D12.

1.5.7.2 The skilled person therefore knows the advantages of using tiled or overlapping probes and would do so according to circumstances.

1.5.7.3 Dependent claims 3-26 do not appear to contain any additional features which, in combination with the features of any claim to which it refers, involves an inventive step.

1.5.7.4 The same as for claims 1 and 2 applies mutatis mutandis for independent claim 27 and the claim depending thereon (claim 28).

1.5.8 The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1-28 does not involve an inventive step (Rule 65(1)(2) PCT).