PATENT COOPERATION TREATY

TRANSLATION

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	15.03.2016
Applicant's or agent's file reference P683551P0	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP2015/006291	17.12.2015	16.03.2015

International Patent Classification (IPC) or both national classification and IPC

C12M1/33(2006.01)i, B01L3/02(2006.01)i, C12M1/00(2006.01)i, C12N15/00(2006.01)n, G01N1/00(2006.01)n

Applicant

PANASONIC CORPORATION

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 or 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 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 notified 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/JP	Date of completion of this opinion	Authorized officer
Facsimile No.		Telephone No.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP2015/006291

Box No. I Basis of this 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:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP2015/006291

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

1. Statement

Novelty (N)	Claims	2-4, 7, 9-11	YES
	Claims	1, 5, 6, 8	NO
Inventive step (IS)	Claims	2-4, 7, 9	YES
	Claims	1, 5, 6, 8, 10, 11	NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims		NO

2. Citations and explanations:

Document 1: JP 1995-284674 A (FUJIFILM CORP.) 31 October 1995, examples, paragraphs [0002], [0003], [0019], [0021], fig. 1, 2 (Family: none)

Document 2: 「ヒト iPS 細胞の樹立方法」, CiRA|M&M, [online], 京都大学 物質-細胞統合システム拠点 iPS 細胞研究センター, 2008 年 7 月 4 日, [2016 年 3 月 3 日検索], インターネット <URL:https://www.cira.kyoto-u.ac.jp/j/research/img/protocol/hiPS_Protocol_080703a.pdf>, 第 5 頁-6 頁, 第 13 頁, non-official translation (Methods for establishing human iPS cell lines," CiRA|M&M, [online], Institute for Integrated Cell-Material Sciences, Center for iPS Cell Research and Applications, Kyoto University, 04 July 2008, [retrieval date 03 March 2016], Internet <URL: https://www.cira.kyoto-u.ac.jp/j/research/img/protocol/hiPSProtoco

1080703a.pdf>, pages 5, 6, 13)

Document 3: WO 2014/115799 A1 (TOKYO ELECTRON LTD.) 31 July 2014, example 3-2, paragraphs [0019], [0091], [0094], [0098] & US 2015/0353884 A1 example 3-2, paragraphs [0084], [0158], [0161], [0164] & EP 2949746 A1

Document 4: JP 2014-18185 A (TOKYO ELECTRON LTD.) 3 February 2014, paragraph [0046] & WO 2014/017481 A1

Claims 1, 5, 6, and 8

The invention as set forth in claims 1, 5, 6, and 8 lacks novelty and does not involve an inventive step in the light of document 1 cited in the ISR.

Document 1 discloses a pipette tip to be used when drawing in and retaining a liquid sample such as blood or urine, and then discharging a specified amount of the same. Document 1 further indicates the following: that said pipette tip comprises a liquid housing section 3, an inclined step section 4 and a tip end section 5; that the inner diameter of the draw-discharge aperture 11, which opens into the tip 5b of said tip end section 5, ranges from approximately 0.44 mm to approximately 0.8 mm; and that the length of the tip end section 5 ranges from approximately 2.0 mm to approximately 5.0 mm (paragraph [0002]; examples; fig. 1, 2).

When the invention as in claim 1 of the present application is compared with the invention disclosed in document 1, the "liquid housing section 3" and the "inclined step section 4" correspond to the "main body section" of the invention of the present application, and the "tip end section 5" and "inner diameter of the draw-

discharge aperture 11," respectively, correspond to the "straight pipe section" and "inner diameter of the straight pipe section" of the invention of the present application. Moreover, the 8.0 mm inner diameter of the abovementioned draw-discharge aperture 11 and 5.0 mm length of the tip end section 5 disclosed in document 1, respectively, lie within the numerical ranges for the "straight pipe section" and "inner diameter of the straight pipe section" stipulated in the invention of the present application.

Therefore, the invention set forth in claim 1 does not differ from the pipette tip disclosed in document 1.

Moreover, document 1 states: "In the tip end section 5, a taper is formed such that the inner surface 6d and the outer surface 5a gradually diminish in diameter as they approach the tip 5b ..., and the taper angle $\theta 5$ ranges from 0° (a constant diameter) to approximately 10°" (paragraph [0021]). In addition, document 1 indicates that pipette tips with various shapes are commonly used (paragraph [0003]), and as an example, document 1 cites US 1982-4347875 A (fig. 9), which discloses a pipette tip with a shape having a reverse taper section wherein the inner diameter of the tip end section of the pipette tip increases in diameter toward the tip end.

In addition, a "liquid sample such as blood or urine" disclosed in document 1 will contain cells, and therefore, document 1 discloses a pipetting method for drawing in a liquid that contains cells using the abovementioned pipette tip and discharging the aforementioned drawn in liquid using said pipette tip.

As a result, the invention set forth in claims 5, 6, and 8, which refer to claim 1, does not differ from the pipette tip, etc., disclosed in document 1.

Claims 10 and 11

The invention as set forth in claims 10 and 11 does not involve an inventive step in the light of documents 1-4 cited in the ISR.

As disclosed in documents 2-4, when handling iPS cells, dispersing a cell aggregate by pipetting was common practice before the priority date of the present application (document 2: page 13, document 3: paragraph [0019], document 4: paragraph [0046]). Regarding the tip, etc., used for pipetting in document 2, the following is stated: "Equivalent equipment and materials sold by other manufacturers can be substituted" (pages 5, 6), and therefore it can be said that a commonly used pipette tip can also be utilized for dispersing cell aggregates by pipetting as disclosed in documents 2-4.

This being the case, based on the common practice disclosed in documents 2-4, a person skilled in the art could easily conceive of using the kind of abovementioned pipette tip disclosed in document 1, which can be used for a liquid sample such as blood or urine, to disperse cell aggregates when pipetting a liquid that contains human iPS cells.

Moreover, regarding human iPS cells, document 3 (example 3-2) states: "From the viewpoint of spreading properties, preferably the average number of [subcultured] cells per individual cell aggregate is less than or equal to 1,209 cells (corresponding to a diameter of 216 μm) and more preferably less than or equal to 867

cells (corresponding to a diameter of 195 μm)" (paragraph [0094]), and "When the number of cells per individual cell aggregate is 55-217 cells, a proliferation ratio greater than or equal to 1/2 of the maximum cell proliferation ratio can be expected" (paragraph [0098]). Based on the disclosures of document 3 (paragraph [0091]), when the abovementioned number of cells is 55-217 cells, the diameter of a cell aggregate is 69-114 μm. Therefore, to prepare iPS colonies of a suitable shape during subculturing and growth, a person skilled in the art could, as appropriate, refer to the disclosures of document 3 and break up the iPS colonies so that the diameter lies between 100 μm and 200 μm when performing a pipetting procedure derived from documents 1-4 on the abovementioned liquid containing human iPS cells.

Meanwhile, the invention as set forth in claims 10 and 11 covers methods wherein the pipetting conditions are unspecified, and it cannot be said that the entirety of the same exhibits an advantageous effect.

Claims 2-4, 7, and 9

The invention as set forth in claims 2-4, 7, and 9 has novelty and involves an inventive step in relation to documents 1-4 cited in the ISR.

As noted above, document 1 discloses the abovementioned pipette tip that is used for drawing in and discharging a liquid containing cells, and documents 2-4 disclose common practice wherein cell aggregates are dispersed by pipetting when handling iPS cells, etc. However, documents 1-5 do not disclose or suggest the following matters: that "The aforementioned diameter of the aforementioned straight tube section is 1.0-1.5 mm,

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

inclusive" as set forth in claim 2; that "The interior wall of the aforementioned straight tube section is disposed unevenly" as set forth in claim 7; and that "5-12 mL, inclusive, of the aforementioned liquid is drawn and discharged 2-5 times, inclusive, at a rate of 3.0-7.0 mL/sec, inclusive" as set forth in claim 9. Furthermore, it can be said that a person skilled in the art could not easily conceive of said matters.