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Datasheet for the decision of 24 January 2023

Case Number: T 1705/18 - 3.3.08

Application Number: 10762102.1

Publication Number: 2414548

IPC: C12Q1/68, C12N15/11, C40B50/06

Language of the proceedings: EN

Title of invention:
GENE EXPRESSION ANALYSIS IN SINGLE CELLS

Patent Proprietor:
Illumina, Inc.

Opponents:
Strawman Limited
Vossius & Partner
Patentanwälte Rechtsanwälte mbB
Vogel /Bals, Andreas / Rüdiger

Headword:
Gene expression analysis in single cells/ILLUMINA

Relevant legal provisions:
EPC Art. 56

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It can be changed at any time and without notic

Keyword:
Main request and auxiliary requests 1 to 3 - inventive step (no)

Decisions cited:

Catchword:

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Case Number: T 1705/18 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 24 January 2023

Appellant: (Opponent 1)	Strawman Limited Orchard Lea Horns Lane Combe, Witney Oxfordshire OX29 8NH (GB)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted on 24 April 2018 concerning maintenance of the European Patent No. 2414548 in amended form**

Composition of the Board:

Chair T. Sommerfeld
Members: D. Pilat
R. Winkelhofer

Summary of Facts and Submissions

I. European patent No. 2 414 548 is based on European patent application No. 10 762 102.1, originally filed as international patent application published as WO 2010/117620. The patent was opposed on the grounds of Article 100(a) in conjunction with Articles 54 and 56 EPC, and of Articles 100(b) and (c) EPC.

II. Opponents O1 and O2 (appellants I and II) lodged appeals against the interlocutory decision of an opposition division to maintain the patent on the basis of the main request filed on 15 January 2018. With the statement of grounds of appeal, appellant I submitted new documents D42 and D43.

III. The patent proprietor (respondent) replied to the appeals, upheld the main request and submitted auxiliary requests 1 to 3.

IV. Appellant I submitted further documents D44 to D52.

V. The party as of right (opponent 3) made no substantive submissions.

VI. The board summoned the parties to oral proceedings and sent a communication pursuant to Article 15(1) RPBA, providing its provisional opinion on some issues.

VII. Oral proceedings took place on 24 January 2023, in the absence of appellant I and opponent 3, who had informed in advance that they would not be represented at oral proceedings.

VIII. Claim 1 of the **main request** reads as follows:

"1. A method of preparing a cDNA library from a plurality of single cells, the method comprising the steps of:

(i) releasing mRNA from each single cell to provide a plurality of individual mRNA samples, wherein the mRNA in each individual mRNA sample is from a single cell;

(ii) synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample and incorporating a distinct tag or a distinct combination of tags into each individual cDNA sample to provide a plurality of tagged cDNA samples, wherein each cDNA sample has a distinct tag or combination of tags, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell;

(iii) pooling the tagged cDNA samples; and

(iv) amplifying the pooled cDNA samples to generate a cDNA library comprising double-stranded cDNA."

Claim 1 of **auxiliary request 1** differs from claim 1 of the main request in that step (ii) further stipulates that "... the tag is incorporated into the cDNA during its synthesis".

Claim 1 of **auxiliary request 2** differs from claim 1 of the main request in that the pooling of the tagged cDNA samples in step (iii) occurs "...prior to any amplification step".

Claim 1 of **auxiliary request 3** is identical to claim 1 of the main request.

IX. The documents cited in this decision include the following:

D1: Kurimoto, K. *et al.*, Nucleic Acids Research, vol. 34(5),e42, pages 1 to 17 (2006);
D2: Makrigiorgos, G.M. *et al.* Nature Biotechnology, vol. 20, pages 936 to 939, 2002;
D17: Qiu, F. *et al.*, Plant Physiology, vol. 133, pages 475 to 481, 2003;

X. The parties' submissions, insofar as they are relevant for this decision, are discussed in the Reasons, below.

XI. Appellants I and II request that the decision of the opposition division be set aside and amended such that the patent be revoked. Appellant II further requests that auxiliary request 3 be not admitted and considered in the appeal proceedings.

XII. The respondent requests that the appeal be dismissed (main request) or that the patent be maintained on the basis of auxiliary requests 1, 2 or 3, all filed with the reply to the appeal.

Reasons for the Decision

Main request
Inventive step (Article 56 EPC)

Closest prior art

1. According to the patent, the purpose of the claimed invention is to provide cDNA libraries from single cells, which can be used to analyse gene expression in a plurality of single cells (patent paragraph [009]). The method allows identification of natural variations in gene expression on a cell by cell level, with reduced amplification bias and enabled back-tracing of

the analysed cDNAs to a single cell by using individual tags (paragraphs [0012], [0014], [0015] of the patent).

1.1 Document D2, which also discloses the production of traceable cDNA libraries and therefore relates to the same or at least a similar purpose as the claimed invention, is an appropriate closest prior art document based on which a problem-solution approach can be elaborated, even if the respondent contended that document D2 was not the closest prior art due to its differences, and thus not close enough compared to another "closer" prior art document D1. A document selected by a party as a starting point for assessing inventive step cannot be excluded only because some seemingly more promising item of prior art is available (T 405/14, Reasons 19). If the skilled person has a choice of several pieces of prior art which might lead to the invention, the rationale of the problem-solution approach requires that the invention be assessed relative to all these possible pieces of closest prior art, before an inventive step can be acknowledged (e.g. T 967/97, Reasons 3.2; T 21/08, Reasons 1.2.3; T 1742/12, Reasons 6.6; T 1012/19, Reasons 22). Should the invention be obvious to the skilled person from at least one of the prior art documents, in this case from document D2, then no inventive step can be acknowledged anyway.

Objective technical problem and solution

1.2 Document D2 concerns a technique called "balanced PCR" capable of avoiding PCR amplification bias when carrying out genetic analysis including expression profiling on microarrays (abstract). Thus, like the patent, document D2 addresses the problem of avoiding

or reducing the amplification bias when separate samples are amplified by PCR amplification.

1.3 Document D2 provides a solution to the problem of bias introduced by PCR amplification. In particular, it describes a genome analysis of two distinct genomic DNA samples tagged with oligonucleotides containing both a common and a unique DNA sequence and a restricted gene expression analysis of lung and prostate-derived amplified cDNAs using the "balanced PCR" method in both settings (abstract, left-hand column, first sentence; page 936, right-hand column; page 938 legend of Figure 2). Document D2 provides a proof of principle for the application of the "balanced PCR" method to digested genomes or cDNAs: "[t]he principle of this method has been validated with synthetic DNA, genomic DNA, and cDNA applied on microarrays" (page 936, abstract, right-hand column, lines 15 to 17). Moreover, the "balanced PCR" method is indicated to be applicable to single cells and to gene expression analysis (abstract, last sentence).

1.4 The board agrees with the respondent that the differences between the method of claim 1 and document D2 consist of steps (i) to (iv) not being disclosed in document D2. In other words, document D2 fails to provide a detailed protocol on how to apply the "balanced PCR" method on individual cells when differential gene expression analysis is of interest. The effect linked to these differences is that cDNA libraries can be prepared from single cells which can then be used for gene expression analysis applications.

1.5 Thus, starting from document D2, in line with appellant II's arguments, the technical problem is how to put into practice the "balanced PCR" method to single cells

to enable their genetic analysis, e.g. their differential gene expression, as explicitly suggested in document D2. This technical problem is also in line with the respondent's formulation as being to provide a library of cDNA samples derived from a plurality of single cells, which can be used for gene expression analysis that can be attributed to the single cell, the method being without amplification bias. The method according to claim 1 solves this problem.

Obviousness

1.6 It remains to be assessed whether or not the skilled person, starting from document D2 and faced with the problem identified above, would have arrived at the method according to claim 1 in an obvious manner.

1.6.1 First, the skilled person was motivated to apply the method of document D2 also for gene expression analysis, as is explicitly suggested in document D2 (abstract, supra). Second, they would know that a differential gene expression applied to a plurality of single cells requires the preparation of a cDNA library, as defined in paragraph [0018] of the patent, which is obtained by distinctly tagging the cDNA synthesised from mRNA released from each individual cell, pooling the labelled cDNA samples and amplifying said pooled tagged cDNA samples.

1.6.2 Although document D2 fails to directly disclose how cDNA is produced from single cells, the release of mRNA from single cells and the use of reverse transcription are standard steps for cDNA production well known in the art. As to the second step of the method of claim 1 consisting of synthesizing a first strand of cDNA ... and incorporating a distinct tag or a distinct

combination of tags into each individual cDNA sample, it does not exclude the step of incorporating a distinct tag by ligation when applying the broadest meaningful definition to the term "incorporating". Incorporation of a distinct tag by ligation is also disclosed in document D2, so that the source material from each individual source can be identified. The following steps of pooling the cDNA samples and of amplifying the pooled cDNA samples in the "balanced PCR" method are routine steps for the skilled person. Thus, the claimed solution is obvious.

1.7 The respondent asserted that the "balanced PCR" method disclosed in document D2 was only applied to two tagged genomic DNA samples and two commercially available cDNA libraries from organ tissues, and thus exemplified for double-stranded DNA samples only. Even if document D2 referred to expression profiling and single cells in the abstract, this was only a suggested possibility among others, and document D2 did neither disclose nor suggest the preparation of a cDNA library from single cell for gene expression profiling. It even less disclosed a tag being incorporated into the first strand of a cDNA synthesized from the mRNA in each individual mRNA sample resulting in an mRNA/cDNA-hybrid.

1.8 The board notes that document D2 clearly suggested applying the "balanced PCR" method to single cells for gene expression analysis, and, as argued above, the skilled person would thus just have to routinely adapt the method disclosed in document D2 to this particular application. The respondent's arguments are thus not persuasive.

1.9 Consequently, the main request lacks an inventive step (Article 56 EPC).

Auxiliary request 1- Inventive step (Article 56 EPC)

2. In claim 1 of auxiliary request 1, a limitation was introduced into step (ii), namely "wherein the tag is incorporated into the cDNA during its synthesis".

2.1 Starting from the closest prior art document D2, a technical effect cannot be attributed to said limitation when introduced into the method of claim 1. Thus, the skilled person confronted with the technical problem above would have turned to document D17 which discloses that the first-strand cDNA synthesis is primed with primers containing a distinguishable barcode sequence (page 480, right-hand column, first full paragraph), and would therefore have arrived at the method of claim 1. This solution represents at best an obvious and consequently non-inventive choice among all the known and equally likely possibilities that solve the problem posed.

2.2 The rationale for the lack of inventive step for claim 1 of the main request is not affected by the amendment introduced in claim 1 of the main request, with the consequence that also this request does not involve an inventive step.

Auxiliary request 2 - Inventive step (Article 56 EPC)

3. In claim 1 of auxiliary request 2 another limitation was introduced, namely that the pooling of the tagged cDNA samples in step (iii) occurs "... prior to any amplification step".

3.1 Document D2 already included a step of pooling the tagged cDNA samples prior to any amplification step, therefore this is not a further distinguishing feature. The rationale for the lack of inventive step for claim 1 of the main request is not affected by the amendment introduced in claim 1 of the main request, with the consequence that also this request does not involve an inventive step.

Auxiliary request 3 - Inventive step (Article 56 EPC)

4. Claim 1 of auxiliary request 3 is identical to claim 1 of the main request. Hence, for the same reasons as for the main request, auxiliary request 3 does not involve an inventive step either.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chair:

L. Malécot-Grob T. Sommerfeld

Decision electronically authenticated