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Datasheet for the decision of 7 April 2011

Case Number: T 1739/07 - 3.3.08

Application Number: 97922283.3

Publication Number: 0920440

IPC: C07H 21/04

Language of the proceedings: EN

Title of invention:
Detection of nucleic acid sequence differences using the ligase detection reaction with addressable arrays

Applicant:
CORNELL RESEARCH FOUNDATION, INC., et al

Headword:
Addressable array/CORNELL RESEARCH FOUNDATION

Relevant legal provisions:
EPC Art. 123(2), 84, 54, 56, 83

Relevant legal provisions (EPC 1973):
-

Keyword:
"Main request meets requirements of the EPC"

Decisions cited:
-

Catchword:
-

Case Number: T 1739/07 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 7 April 2011

Appellant: (Applicant)
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Decision under appeal:
Decision of the Examining Division of the European Patent Office posted 7 May 2007 refusing European patent application No. 97922283.3 pursuant to Article 97(1) EPC 1973.

Composition of the Board:

Chairman: M. Wieser
Members: M. R. Vega Laso
J. Geschwind

Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division posted on 7 May 2007, by which the European patent application No. 97 922 283.3 with the title "Detection of nucleic acid sequence differences using the ligase detection reaction with addressable arrays" was refused under Article 97(1) EPC 1973.

II. In the decision under appeal, the amended claims according to the main request filed during oral proceedings on 23 March 2007 were found to conform to Article 123(2) EPC 1973, and their subject-matter to fulfil the requirement of novelty (Article 54 EPC 1973). However, the subject-matter of claim 1 was considered to lack an inventive step within the meaning of Article 56 EPC 1973.

For the assessment of inventive step, document (1) was regarded as the closest prior art. In the view of the examining division, this document described a method of multiplex ligation-dependent amplification/detection of multiple target nucleic acids for the detection of variations (e.g. mutations, deletions, etc) in target nucleic acids present in a sample. The examining division held that, having regard to the content of document (1), the technical problem to be solved could be formulated as the provision of an alternative means of detection of the products obtained by ligase chain reaction. The solution provided in claim 1 was a method in which the probes were equipped with oligonucleotide tails which allowed the detection/separation of the amplification products on spatially addressable oligonucleotide arrays.

The examining division considered that document (1) already suggested the use of polynucleotide tails to achieve separation of the amplification products, and that in documents (3) and (4) spatially addressable arrays which allowed the detection of products equipped with polynucleotide tails were described. Moreover, at the priority date, a person skilled in the art was well aware of the design requirements for probe or polynucleotide tails in order to avoid cross- or unspecific hybridisation and to improve hybridisation conditions. Even though the examining division acknowledged that the features of the method of claim 1 characterising the capture oligonucleotides on the addressable array were not apparent from document (1), it held that the features in question did not solve any unforeseeable technical problem, but represented only obvious design measures. Thus, the solution provided in claim 1 of the main request was regarded as obvious in view of a combination of document (1) and document (3) or (4).

Similar reasons were given by the examining division for its adverse finding on inventive step in respect of claim 1 of each of the auxiliary requests 1 to 4 filed during the oral proceedings.

III. In their statement of grounds of appeal, the appellants (applicants) pursued further the sets of claims according to the main request and the auxiliary requests 1 to 3 on the basis of which the application had been refused, but withdrew auxiliary request 4. Together with the statement of grounds, further evidence was submitted.

IV. The examining division did not rectify its decision and referred the case to the board of appeal under Article 109(2) EPC 1973.

V. The appellants were summoned to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons, the board informed the appellants of its preliminary, non-binding opinion on some of the issues to be discussed at the oral proceedings, in particular issues concerning Articles 123(2), 84 and 56 EPC.

VI. As a reply to the board's communication, the appellants withdrew their previous requests and filed five new sets of claims as main request and auxiliary requests 1 to 4, respectively.

VII. During the oral proceedings, which were held on 7 April 2011, the appellants filed a set of amended claims as a fresh main request which replaced the main request then on file.

VIII. The set of claims of the appellants' main request consists of 38 claims. Independent claim 1 reads as follows:

"1. A method for identifying one or more of a plurality of target sequences, the target sequences differing from each other by one or more single-based changes, insertions, deletions, or translocations, comprising:

providing a sample potentially containing one or more target nucleotide sequences with one or a plurality of sequence differences;

providing a plurality of oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe, having a target-specific portion and an addressable array-specific portion and (b) a second oligonucleotide probe, having a target-specific portion and a detectable reporter label, wherein the oligonucleotide probes in a particular set are suitable for ligation together when hybridized adjacent to one another on a corresponding target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample;

providing a ligase;

blending the sample, the plurality of oligonucleotide probe sets, and the ligase to form a mixture;

subjecting the mixture to one or more ligase detection reaction cycles comprising a denaturation treatment, wherein

any hybridized oligonucleotides are separated from the target nucleotide sequences, and a hybridization treatment, wherein the oligonucleotide probe sets hybridize at adjacent positions in a base-specific manner to their corresponding target nucleotide sequences, if present in the sample, and ligate to one another to form a ligated product sequence containing (a) the addressable array-specific portion, (b) the target-specific portions connected together, and (c) the detectable reporter label, and, wherein the oligonucleotide probe sets may hybridize to nucleotide sequences in the sample other than their respective target nucleotide sequences but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment;

providing a solid support with different capture oligonucleotides immobilized at particular sites, wherein the capture oligonucleotides have nucleotide sequences complementary to the addressable array-specific portions and, wherein the solid support and the capture oligonucleotides form an addressable array;

contacting the mixture, after said subjecting, with the solid support under conditions effective to hybridize the addressable array-specific portions to the capture oligonucleotides in a base-specific manner, thereby capturing the addressable array-specific portions of the solid support at the site with the complementary capture oligonucleotide; and

detecting the reporter labels of ligated product sequences captured to the solid support at particular sites, thereby indicating the presence of one or more target nucleotide sequences in the sample, wherein

(a) the capture oligonucleotide sequences do not hybridize to the target sequences at high stringency, and

(b) all the capture oligonucleotides on the solid support have similar Tms so as to hybridize specifically to complementary addressable array specific portions under uniform hybridization conditions at high stringency."

Dependent claims 2 to 33 concern particular embodiments of the method of claim 1. Independent claim 34 and dependent claims 36 to 38 are directed to a kit comprising specific reagents and a solid support for performing the method according to claim 1. Claim 35 is directed to a further particular embodiment of either the method of claim 1 or the kit of claim 34.

IX. The following documents are cited in the present decision:

D1: WO 93/20227 (published on 14 October 1993);

D3: WO 93/17126 (published on 2 September 1993);

D4: US 5412087 (published on 2 May 1995).

X. The submissions of the appellants with respect to the issue of inventive step can be summarised as follows:

Starting from document (1) as the closest prior art, the problem to be solved should be formulated as the provision of an alternative means of detecting by means of a solid support, ligated products produced by a ligase detection reaction (LDR) which indicate the presence of a particular target DNA in the sample analysed. While the possibility of using polynucleotide tails was mentioned in document (1), the teaching of this document was restricted to the use of oligonucleotides labelled with haptens and immobilised antibodies to capture haptenated ligation products. Document (1) did not suggest using oligonucleotides with a separate oligonucleotide addressable array-specific portion, nor that the capture oligonucleotide probes should have no homology to the target sequences, or that the capture oligonucleotide probes on the solid support should hybridise to complementary addressable array-specific portions under uniform hybridisation conditions. Instead, document (1) taught that the immobilised (capture) oligonucleotides should be specific for sequences found in the target sequence.

In document (1), polynucleotide tails were discussed in terms of their use as an indirect label for detection purposes and not in respect of separation or discriminatory hybridisation. Moreover, there was no discussion regarding optimum conditions for sequence specific hybridisation between the polynucleotide tail and the signalling entity complex.

XI. The appellants requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request filed during the oral proceedings.

Reasons for the Decision

Main request - Articles 123(2) and 84 EPC

1. The board is satisfied that the amendments introduced into claims 1 to 38 conform to Article 123(2) EPC.

2. Present claim 1 is derived from claim 1 of the application as filed. The amendments introduced into the claim are based on the following passages of the application as filed:

   i) the additional feature "*... wherein the solid support and the capture oligonucleotides form an addressable array;*" can be derived already from claim 1 of the application as filed, particularly in view of Figures 3 to 10. The method defined in claim 1 of the application as filed comprises the step of "*... providing a solid support with different capture oligonucleotides immobilized at particular sites, wherein the capture oligonucleotides have nucleotide sequences*

complementary to the addressable array-specific portions [of the first oligonucleotide probes];..." (the same wording is included in present claim 1; see paragraph VIII above). While in claim 1 of the application as filed it is not expressly indicated that the solid support and the capture oligonucleotides form an addressable array, it is apparent from each of Figures 3 to 10, which illustrate several embodiments of the method according to the invention, that the fluorescent products resulting from the previous ligation step are captured "*on addressable array*"(see legend for step 3 in each of Figures 3 to 10). The figures show a solid support with capture oligonucleotides attached to it, as well as first oligonucleotide probes which hybridise to the capture oligonucleotides, some of the first oligonucleotide probes being ligated to labelled second oligonucleotide probes;

ii) the additional feature "*... the capture oligonucleotide sequences do not hybridize to the target sequences at high stringency, ...*" is implicit in the passage on page 24, lines 9 to 11 of the application as filed ("*... A capture oligonucleotide probe sequence does not have any homology to either the target sequence or to other sequences on genomes which may be present in the sample ...*"); and

iii) the feature "*... all the capture oligonucleotides on the solid support have similar Tms so as to hybridize specifically to complementary addressable array specific portions under uniform hybridization conditions at high stringency*" can be derived from the passages on page 35, lines 12 to 15 (similar Tms); page 13, lines 11 and 12; page 49, lines 16 to 18 (specific hybridisation to complementary sequences under uniform hybridisation conditions); and page 23, lines 11 and 12, sentence bridging pages 24 and 25 (at high stringency).

The further amendments to claim 1 are regarded by the board as editorial amendments which have been introduced for the sake of either clarity - as in the case of the amendment introduced into the preamble ("*..., **the target sequences** differing **from each other** by ...*") - or consistency, e.g. the amendment introduced into the first step of the method to bring the wording into line with the wording of the preamble ("*... target nucleotide sequences with **one or** a plurality of sequence differences; ...*")(emphasis added by the board).

3. Dependent claims 2 to 5 correspond, respectively, to claims 4 to 7 of the application as filed.

Claim 6 is directed to two alternative embodiments of the method of claim 1 which correspond to the subject-matter of claims 9 and 10 of the application as filed.

Claims 7 to 11 correspond, respectively, to claims 11, 12, 14, 24 and 28 of the application as filed.

Claim 12 is directed to subject-matter claimed in claims 45, 46 and 50 of the application as filed.

The subject-matter of claim 13 was claimed in claims 47 and 49 of the application as filed.

Claims 14 to 16 correspond, respectively, to claims 48, 51 and 52 of the application as filed.

Claim 17 is derived from claim 57 of the application as filed, which has been amended by incorporation of the particular genetic diseases specified in claim 58 of the application as filed.

Claims 18 to 23 correspond, respectively, to claims 59, 61, 75, 79, 80 and 83 of the application as filed.

Claim 24 is derived from claim 84 of the application as filed, which has been amended to specify, as the preferred configuration, a method with the features in claim 85 of the application as filed.

Claims 25 to 31 correspond, respectively, to claims 87, 91, 92, 94, 95, 97 and 99 of the application as filed.

Claim 32 is derived from claim 101 of the application as filed which has been amended to incorporate the features specified in claim 104 of the application as filed.

Claim 33 corresponds to claim 111 of the application as filed.

Independent claim 34 is derived from claim 138 of the application as filed, which has been amended, *mutatis mutandis*, essentially in the same manner as claim 1 (see paragraph 2 above).

Claims 35 to 38 correspond, respectively, to claims 143, 144, 145 and 147 of the application as filed.

4. Amended claims 1 to 38 are considered to be clear and concise, and meet the requirements of Article 84 EPC.

Sufficiency of disclosure (Article 83 EPC)

5. The examining division did not raise any objection under Article 83 EPC. In view of the facts and evidence on file, the board has no reason to doubt that the application discloses the claimed invention in a manner sufficiently

clear and complete for it to be carried out by a person skilled in the art. Thus, the requirement of Article 83 EPC is fulfilled.

Novelty (Article 54 EPC)

6. In the decision under appeal, the examining division did not raise an objection of lack of novelty in respect of the claims then on file. As concerns the present claims, the board is satisfied that the requirement of Article 54 EPC is fulfilled.

Inventive step (Article 56 EPC)

7. Document (1) is regarded as the closest state of the art for the assessment of inventive step, applying the problem-solution approach.

8. Document (1) describes a method of multiplex ligation chain reaction (LCR) for amplifying and detecting multiple putative target nucleic acids in a sample, e.g. possible alternatives of an allele (see page 6, second paragraph, in particular line 15). The method comprises: (i) providing a reaction solution that contains nucleic acid from a sample having one or more of a plurality of target nucleic acid sequences; (ii) providing a probe set having four nucleic acid probes for each putative target sequence: a first and a second probe hybridising to adjacent segments of a target nucleic acid are then joined to form a reorganised primary molecule, while the third and the fourth probes hybridising to adjacent portions of the reorganised primary molecule are joined to form a reorganised secondary molecule; (iii) amplifying the reorganised primary and secondary molecules by cycles of hybridisation/ligation/denaturation; (iv) detecting the amplification product by means of a unique detectable label associated with each probe set.

9. The preferred method for joining the probes in step (ii) uses a thermostable ligase (see page 6, lines 16 to 18).

10. The labels associated with each probe set may be specific binding members such as haptens or polynucleotides (see, *inter* alia, the passage from page 2, line 32 to page 3, line 32; also page 7, lines 12 to 14 and lines 20 and 21). They are used for either detection or separation, or both (see page 3, line 32). In a preferred embodiment of the method described in document (1), each of the probe sets is labelled with two distinct labels, at least one being unique and the other being a common label (see page 3, lines 32 to 36).

11. In a second configuration of the method described in document (1), the probe set for each putative target sequence consists of at least two, <u>optionally</u> four, nucleic acid probes (see passage from page 4, line 6 to page 5,

line 7). The same variations of labelling, separation and detection described for the first configuration are said to be useful (see page 5, lines 8 and 9). Thus, as regards the number of probes in each probe set, the board cannot identify any difference between the method described in document (1) and that of claim 1, because in the latter each probe set does not necessarily consist exclusively of a first and a second probe, but is merely characterised by the two probes.

12. In the method described in document (1), separation of the labelled products may be accomplished by immobilising a specific binding partner for each specific binding member at a different location on a single solid phase (see claim 12 in document (1)). If a polynucleotide is used as the specific binding member for the separation of the ligation products, a hybridisation probe specific for sequences in either the primary or the secondary molecule obtained by ligation is immobilised on the solid phase (see passage bridging pages 11 and 12 of document (1)). The hybridisation probes suggested in document (1) are, thus, equivalent to the "capture oligonucleotides" in present claim 1.

13. In view of document (1), the objective technical problem to be solved can be formulated as the provision of a more reliable method for high-throughput separation and detection of the ligation products which is less prone to false-positive or false-negative results.

14. According to the method of present claim 1, this problem is solved by providing capture oligonucleotides immobilised at particular sites of a solid support forming a spatially addressable array. The capture oligonucleotides have similar Tms so as to hybridise specifically to complementary addressable array specific portions of the ligation products under uniform hybridisation conditions at high stringency, but do not hybridise to the target sequences at high stringency. This allows the selective capture of each ligation product at a specific address on the solid support under the same hybridisation conditions.

15. The board is persuaded that the method claimed in claim 1 does overcome the problems associated with false positive and false negative results in direct hybridisation arrays for ligation products as described in document (1). By relying on divergent capture oligonucleotide sequences which do not hybridise, at high stringency, to the target nucleic acid sequences, and which have similar melting temperatures, uniform high stringency hybridisation conditions can be used which allow for a maximum of specific hybridisation between complementary sequences with minimisation of unspecific cross-hybridisation. While it is conceivable that non-ligated products may also bind to the immobilised capture oligonucleotides of the array, no signal is produced because the detectable label is missing. The production of a signal

in the array requires the presence of the ligation product, which is the only product having both an addressable-array specific portion and a detectable label. The claimed method thus allows for simultaneous and reliable target identification and discrimination of closely spaced and overlapping mutations, including small insertions and deletions, without generating false-positive or false-negative signals.

16. Contrary to the examining division's view, the board judges that, even though the features of the claimed method may have been separately described in different documents cited in the decision under appeal, in particular documents (1), (3) and (4), the combination of the features of claim 1 leading to the advantages of the present invention is not derivable from these documents.

17. While it is true that document (1) suggests that the ligation products can be separated and/or detected by sequence-specific probe hybridisation instead of haptens (see page 11, lines 35 and 36), the sole technical teaching provided in document (1) concerning the suggested immobilised probes is that they must be specific for sequences found in the ligation products (see page 12, lines 2 to 6). There is, however, no indication of how to design such probes in order to achieve a reliable separation and detection of the ligation products and avoid false positives which may result from the hybridisation of target molecules with a sequence which may be, at least in part, similar or identical to the sequence of the corresponding ligation product. Nor is there any suggestion of how to design an array with multiple hybridisation probes differing from each other so as to avoid cross-hybridisation between the ligation probes, while, at the same time, keeping the hybridisation conditions as simple and practical as possible. It should be noted that the examples provided in document (1) merely describe the immunochromatographic separation of ligation products derived from seven different oligonucleotide probe sets, using as specific binding partners seven antibodies immobilised on a nitrocellulose strip, each antibody recognising one of seven different haptens linked to the ligation products (see, *inter alia*, Examples 6 and 10).

18. The board also disagrees with the examining division's finding that the method of claim 1 was obvious in view of a combination of document (1) and either document (4) or (3). The examining division held that, at the priority date of the application, a person skilled in the art would have known from either document that a product equipped with a nucleotide tail as suggested in document (1) could be detected using a spatially addressable array.

19. Document (4) in fact describes a spatially addressable array of probes immobilised on the surface of a substrate which is

used to perform multiple parallel hybridisation reactions. It is stated in document (4) that this system allows the design of the capture sequences without constraint from the target sequence, thus making it possible to perform assays for many different target nucleic acids simultaneously. It is apparent from the passage of document (4) indicated in the decision under appeal (column 11, lines 13 to 35) that, in order for a target sequence to be "captured" on the array, a further probe ("capture probe") is required in the reaction solution, i.e. not attached to the solid support, this probe having a sequence which is in part complementary to the target sequence to be detected, and in part complementary to an immobilised probe. Thus, the hybridisation probes immobilised on the solid support (which are equivalent to the "capture probes" according to present claim 1) do not hybridise to the target sequences.

20. The board accepts that there was no reason for a person skilled in the art at the priority date to doubt that the detection method described in document (4) could work in combination with the method of document (1), and that a skilled person could and would consider combining the teachings of the two documents. However, by combining the two documents he or she would not have arrived at a method as defined in claim 1, but at a different method requiring, for each putative target sequence, an additional probe which hybridises to both the ligation product and the hybridisation probe immobilised on the solid support, as described in document (4). By contrast, such an additional probe is not necessary in the method of claim 1, because in the claimed method the addressable array-specific portion of a ligation product hybridises directly to a particular capture probe immobilised on the support. Thus, contrary to the examining division's finding, the board considers that the method of claim 1 is not obvious to a person skilled in the art in view of a combination of the teachings of documents (1) and (4).

21. In its decision, the examining division also pointed to the passage from page 8, line 23 to page 9, line 31 of document (3). This document relates to oligonucleotide arrays and their use for sorting, isolating, sequencing and manipulating nucleic acids, and the passage in question describes how mixtures of nucleic acid strands can be sorted on a binary array according to either their terminal oligo segments ("terminal sorting") or their internal oligo segments ("internal sorting"). In the sorting method described in document (3), each strand can be provided with universal terminal priming regions that enable PCR amplification without prior knowledge of the terminal nucleotide sequences and without the need to synthesise individual primers. As regards terminal sorting, it is stated that "*... the priming region(s) can be made essentially dissimilar from the sequences occurring in the nucleic acids that are present in the mixture to be sorted, so that priming does not occur anywhere but at the strands'*

termini." (see sentence bridging pages 8 and 9). Since the priming region(s) are the segments which hybridise to specific locations on the array, a person skilled in the art would infer from this passage that the aim is to avoid the target sequences themselves (i.e. the sequences occurring in the nucleic acid present in the mixture) hybridising to the array.

22. However, a person skilled in the art could not find in document (3) any indication that the capture oligonucleotides on the solid support must have similar Tms so as to hybridise specifically to the complementary addressable array-specific portions under uniform hybridisation conditions at high stringency. While it may be true that - as the examining division stated - it is part of the common general knowledge in the field that hybridisation probes, e.g. for amplification by PCR, which are to be used in the same reaction should be designed to have the same thermodynamic properties, so as to enable reaction conditions which are optimal for all probes to be used, the situation in the present case is different. The skilled person is confronted with the problem of designing a large number of capture probes which must be dissimilar in order to avoid cross-hybridisation, and also different from the target sequences.

23. For these reasons, the board comes to the conclusion that the method of claim 1 involves an inventive step. The same reasoning applies, *mutatis mutandis*, to the kit according to independent claim 34, which includes an addressable array with capture oligonucleotides having the features specified in claim 1.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to grant a patent on the basis of the main request, claims 1 to 38 filed during the oral proceedings, and a description to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski M. Wieser