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Datasheet for the decision of 2 March 2016

Case Number: T 0014/12 - 3.3.08

Application Number: 04706051.2

Publication Number: 1594980

IPC: C12N15/10, C12Q1/68, C07H21/00

Language of the proceedings: EN

Title of invention:
BEAD EMULSION NUCLEIC ACID AMPLIFICATION

Patent Proprietor:
454 Life Sciences Corporation

Opponent:
Life Technologies Corporation

Headword:
Emulsion asymmetric PCR pre-hybridization single stranded template immobilized primer bead/454 LIFE SCIENCES

Relevant legal provisions:
EPC Art. 123(2), 88, 56

Keyword:
Main Request - admissibility (yes);
Main Request - meets all requirements EPC (yes)

Decisions cited:

Catchword:

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Case Number: T 0014/12 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 2 March 2016

Appellant: (Opponent)	Life Technologies Corporation 5791 Van Allen Way Carlsbad, CA 92008 (US)
Representative:	Roques, Sarah Elizabeth J A Kemp 14 South Square Gray's Inn London WC1R 5JJ (GB)
Respondent: (Patent Proprietor)	454 Life Sciences Corporation 20 Commercial Street Branford CT 06405 (US)
Representative:	Moore, Jacqueline Frences Cooley (UK) LLP Dashwood 69 Old Broad Street London EC2M 1QS (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 27 October 2011 concerning maintenance of the European Patent No. 1594980 in amended form.**

Composition of the Board:

Chairman	M. Wieser
Members:	P. Julià
	J. Geschwind

Summary of Facts and Submissions

I. European patent no. 1 594 980 is based on European patent application no. 04 706 051.2, published as International patent application WO 2004/069849 (hereinafter "*the application as filed*"). The patent was opposed on the grounds set forth in Articles 100(a), (b) and (c) EPC. The opposition division decided to maintain the patent in amended form on the basis of a Main Request filed on 20 July 2011.

II. The opponent (appellant) lodged an appeal and filed a statement setting out the Grounds of Appeal.

III. In reply to the appellant's Grounds of Appeal, the patentee (respondent) filed a Main Request and three Auxiliary Requests. Except for claim 23, the Main Request was identical to the Main Request upheld by the opposition division. The respondent filed also a "*Second Declaration of Jay Shendure*".

IV. The parties were summoned to oral proceedings and, in a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed thereto, they were informed of the board's preliminary, non-binding opinion on some issues of the case.

V. Both parties replied to this communication informing the board of their attendance to the oral proceedings. The appellant filed an excerpt from the textbook Lodish *et al.*, Molecular Cell Biology.

VI. Oral proceedings were held on 2 March 2016. At these proceedings, the respondent withdrew its Main Request and First Auxiliary Request and made the Second Auxiliary Request its new Main Request.

VII. Claim 1 of the **Main Request** reads as follows:

"1. A method for amplifying one or more nucleic acids onto a bead comprising the steps of:

(a) forming a water-in-oil emulsion to create a plurality of aqueous microreactors wherein at least one of the microreactors comprises one single stranded nucleic acid template, a single bead with a first population comprising a plurality of molecules of a first primer species disposed thereon, the single stranded nucleic acid template being attached to the bead before forming the emulsion, and an amplification reaction solution comprising a second population comprising a plurality of molecules of the first primer species, a plurality of molecules of a second primer species, and reagents necessary to perform nucleic acid amplification, wherein the first primer species is capable of binding to the single stranded nucleic acid template, the second primer species is capable of binding to a complementary strand of the single stranded nucleic acid template, and a concentration of the second primer species is greater than that of the second population of the first primer species, in the amplification reaction solution;

(b) asymmetrically amplifying the single stranded nucleic acid template and the complementary strand to the template strand in the amplification reaction solution, to form a population of amplified copies of the single stranded template nucleic acid, wherein the asymmetric amplification is performed by asymmetric polymerase chain reaction; and

(c) binding a plurality of the asymmetrically amplified copies of the single stranded template nucleic acid to the first population of the first primer species on the bead in the microreactor, wherein a bead bound complementary strand is extended from the first primer species."

Claims 2-13, are directed to preferred embodiments of claim 1. Claims 14 and 23, which comprise all essential features of the method of claim 1, are directed to a method for amplifying a nucleic acid and to a method for producing a clonal population of nucleic acids. Claims 15-22 and 24-29 are directed to preferred embodiments of claims 14 and 23, respectively.

VIII. The following documents are referred to in this decision:

D1: WO-A2-02/103011 (publication date: 27 December 2002);

D7: US-A1-2002/0119459 (publication date: 29 August 2002);

D8: WO-A1-00/40712 (publication date: 13 July 2000);

D9: J.D. Andreadis and L.A. Chrisey, Nucleic Acids Research, 2000, Vol. 28, No. 2, pages i-viii;

D12: D. Dressman et al., Proc. Natl. Acad. Sci. USA, 22 July 2003, Vol. 100, No. 15, pages 8817-8822;

D16: WO-A2-02/22869 (publication date: 21 March 2002).

IX. The submissions of the appellant, insofar as they are relevant to the present decision, may be summarised as follows:

Article 100(c) EPC; Article 123(2) EPC

Claim 1 comprised an embodiment which had no basis in the application as filed, according to which the single stranded nucleic acid template was not attached to the bead through hybridization to the first primer species immobilized on the bead. The claim covered an embodiment wherein the template was attached to the bead by other means, such as chemical linkage. There was no basis in the application as filed for a particular embodiment in which the first primer species was immobilized on the bead and the single stranded nucleic acid template was attached to the bead by a chemical linkage.

Articles 87-89 EPC; Entitlement to priority

The particular embodiment comprised in claim 1 and discussed under Article 123(2) EPC was not disclosed in the priority document US 60/476,504. Therefore, the claims of the Main Request were not entitled to the claimed priority date.

Article 100(a) EPC; Article 56 EPC

Document D9, representing the closest state of the art, disclosed a method that differed from the method of claim 1 by two technical features. The first feature was the use of a single stranded nucleic acid template and the second feature the formation of a water-in-oil (w/o) emulsion (emulsion-PCR) and the attachment of the single stranded nucleic acid template to the bead before forming such emulsion.

As regards the first feature, it had no technical effect because the nucleic acid template, independently of its single or double stranded nature, always became single stranded at the first step of the asymmetric PCR.

As regards the second feature, document D9 referred to the known applications of the methods based on the attachment of PCR amplicons to solid phases and the advances provided by these methods for the development of micro-fabrication and automation strategies for high throughput, compact DNA diagnostic tools. The method disclosed in document D9 was described as being appropriate for these purposes, in particular for the immobilization and subsequent transcription/translation of numerous DNA templates and for the development of micro-fabricated diagnostic devices. Prior art documents on file, such as documents D8, D7, D1 and D16, disclosed the advantageous use of emulsions and emulsions-PCR for methods involving the immobilization and subsequent translation/transcription of DNA templates. Therefore, it was obvious for a skilled person to combine the disclosure of document D9 with anyone of these prior art documents and to arrive at the claimed subject-matter in a straightforward manner.

X. The submissions of the respondent, insofar as they are relevant to the present decision, may be summarised as follows:

Article 100(c) EPC; Article 123(2) EPC

Formal basis for the feature introduced into claim 1 was found on page 5, lines 8-10, and page 7, lines 5-7, of the application as filed. These passages referred to a single stranded nucleic acid template attached to a

capture bead before forming the w/o emulsion. Claim 1 was limited to this "*pre-hybridization*" embodiment which required the single stranded nucleic acid template to be attached to the bead before forming the w/o emulsion and to carry out the asymmetric PCR afterwards. In the light of the entire disclosure of the application as filed and upon a meaningful reading of the features characterizing the method of claim 1, the particular embodiment referred to by the appellant resulted from a misconstruction of the claim.

Articles 87-89 EPC; Entitlement to priority

Claim 1 was directed to the "*pre-hybridization*" embodiment. In the priority document US 60/476,504, this embodiment was disclosed as a preferred embodiment. The Main Request was thus entitled to the claimed priority date.

Article 100(a) EPC; Article 56 EPC

The closest prior art was represented by document D9. The technical problem underlying the patent was the provision of a method that allowed parallel amplification of a large number of nucleic acid templates in isolation from each other. Document D9 was concerned with the amplification of a single nucleic acid template or, at the most, a mixture of two distinct nucleic acid templates, i.e. a very limited, small number of templates. Document D9 did not contain any reference to the amplification of a large number of different nucleic acid templates or of a library of nucleic acid templates. Moreover, the method disclosed in document D9 did not require the isolation of the nucleic acid templates. The introduction of additional steps, such as the formation of an w/o emulsion and

emulsion-PCR, would have added only unnecessary complexity. Therefore, the skilled person had no incentive to combine document D9 with any of the prior art documents D8, D7, D1 and/or D16, all concerned with a different technical problem. Moreover, even if the skilled person would have combined document D9 with any of these prior art documents, it would not have arrived at a method having all features of claim 1 in an obvious manner, as such a combination would not have resulted in a method comprising the use of a single stranded nucleic acid template and the attachment of this template, before forming the w/o emulsion, to the bead through hybridization to a first primer species.

XI. The appellant (opponent) requested that the decision under appeal be set aside and the patent be revoked.

XII. The respondent (patentee) requested that the decision under appeal be set aside and the patent be maintained in amended form on the basis of the Main Request filed at the oral proceedings on 2 March 2016.

Reasons for the Decision

Admissibility of the Main Request

1. The Main Request was originally filed as a "Second Auxiliary Request" in reply to the appellant's statement of Grounds of Appeal and thus, at the earliest stage of the appeal proceedings (cf. points III and VI *supra*). The request is a direct response to, and intends to overcome, the objections raised by the appellant in the statement of Grounds of Appeal. No objection has been raised by the appellant as regards its admissibility and the board has no objections of its own.

Main Request
Article 100(c) EPC; Article 123(2) EPC

2. The application as filed states that "*a single stranded nucleic acid template to be amplified is attached to a capture bead. The template may be captured to the bead prior to emulsification or after the emulsification has been formed*" (cf. page 7, lines 5-7). This passage provides a formal basis for the feature introduced into claim 1 and independent claims 14 and 23.

The attachment of a single stranded nucleic acid template to a capture bead is defined in the passage immediately following as "*mediated by chemical groups or oligonucleotides that are bound to the surface of the bead*" (cf. page 7, lines 8-10), wherein the oligonucleotides "*recognize (i.e., are complementary to) a portion of the nucleic acid template*" (cf. page 8, lines 1-3). Indeed, this corresponds to a first embodiment of the invention in which "*single copies of the nucleic acid template species are hybridized to capture beads*" (cf. page 2, lines 22-24), i.e. the "*pre-hybridization embodiment*".

3. Step (a) of claim 1 requires "*a first primer species*" to be "*disposed"* on the bead and to be "*capable of binding to the single stranded nucleic acid template*" which is "*attached to the bead before forming the emulsion*". Step (c) further requires the "*binding ... of the asymmetrically amplified copies of the single stranded template nucleic acid to ... the first primer species on the bead in the microreactor ...*". Step (b) of claim 1 refers to the asymmetric amplification for which the appropriate concentration ratio of first and second

primer species is defined in step (a) (cf. point VII *supra*).

All these structural and functional features characterize the subject-matter of claim 1 as being limited to the "*pre-hybridization embodiment*" disclosed in the application as filed, in particular on page 5, line 1 to page 6, line 20, with reference to Figure 2. This passage starts by stating that "*bead emulsion amplification is performed by attaching a template (e.g., DNA template) to be amplified to a solid support ... The bead is linked to a large number of a single primer species ... that is complementary to a region of the template DNA ... the template DNA is bound to the bead prior to emulsification ...*" (cf. page 5, lines 1-12). In the light of this information in the application as filed, when reading the claim with a mind willing to understand, a skilled reader will not arrive at an interpretation of the claim, wherein it encompasses, as argued by the appellant, an embodiment in which the first primer species is immobilized on the bead and the single stranded nucleic acid template is attached to the bead by a chemical linkage (cf. point IX *supra*).

4. Therefore, the Main Request fulfils the requirements of Article 123(2) EPC.

Articles 123(3) and 84 EPC

5. Claim 1 of the Main Request differs from claim 1 as granted by the introduction of the feature "*the single stranded nucleic acid template being attached to the bead before forming the emulsion*" in step (a), and by the feature "*wherein the asymmetric amplification is performed by asymmetric polymerase chain reaction*" in

step (b) of claim 1. No objections were raised by the appellant under Articles 84 and 123(3) EPC.

Article 100(b) EPC; Article 83 EPC

6. In the communication pursuant to Article 15 RPBA, the board noted that the objection under Article 100(b) EPC/ Article 83 EPC raised by the appellant in the Grounds of Appeal did not address the respective reasons given by the opposition division in the decision under appeal. The appellant did not further comment on this issue in writing or at the oral proceedings.

7. In the light thereof, the board sees no reason to deviate from the findings of the opposition division on Article 83 EPC. Thus, the Main Request fulfils the requirements of Article 83 EPC.

Articles 87-89 EPC; Entitlement to priority

8. The priority document US 60/476,504 discloses "*a single stranded nucleic template to be amplified is attached to a capture bead*" (cf. page 6, lines 10-11). According to a first embodiment, "*single copies of the nucleic acid template species are hybridized to DNA captured beads*" (cf. page 2, lines 25-30). This first embodiment is described in detail on page 4, line 6 to page 5, line 22 with reference to Figure 2. This passage starts by saying that "*bead emulsion amplification is performed by attaching a template DNA to be amplified to a solid support ... Template DNA annealed to the bead bound primer. The beads are suspended in aqueous reaction mixture and then encapsulated in a water-in-oil emulsion*" (cf. page 4, lines 6-11).

9. In fact, the appellant has not contested that the "*pre-hybridization embodiment*" is disclosed in the priority document, but has argued only that, according to its interpretation of the claim 1 (see the section referring to Article 123(2) EPC, above), the claim is not entitled to the priority date (cf. point IX *supra*). Since the board does not follow appellant's interpretation of claim 1 and considers this claim to be limited to the "*pre-hybridization embodiment*", the Main Request is entitled to the claimed priority date.

Article 100(a) EPC; Article 54 EPC

10. Documents D1 and D12 were the only documents referred to in the decision under appeal.

The decision of the opposition division, that document D1 was not prejudicial for the novelty of claim 1, has not been contested by the appellant in the Grounds of Appeal. Document D12 has a publication date of 22 July 2003, i.e. after the filing of the priority document US 60/476,504 on 6 June 2003. Since the Main Request is entitled to the claimed priority date, document D12 does not belong to the state of the art.

11. Thus, the Main Request fulfils the requirements of Article 54 EPC.

Article 100(a) EPC; Article 56 EPC

12. Document D9 has been identified as the closest prior art document in the decision under appeal. This has not been contested by any of the parties in appeal proceedings. The parties also agreed on the analyses of the opposition division regarding the differences between the subject-matter of the claims underlying the

opposition division and the disclosure in document D9, which were identified as being the fact that the amplification reaction took place in a w/o emulsion and that the template was a single stranded nucleic acid template (cf. page 6, point 2.6.2 of the appealed decision).

13. In addition to the claims before the opposition division, claim 1 of the present Main Request has further been limited in appeal proceedings to the "*pre-hybridization embodiment*" which requires the single stranded nucleic acid template to be attached to the capture bead before forming the w/o emulsion. This feature, which is also not disclosed in document D9, constitutes thus a further difference between the claimed invention and the closest state of the art.

14. The board agrees with the respondent that, starting from document D9, the technical problem to be solved is the provision of a method for parallel amplification of a high number of templates simultaneously and in isolation from each other. The patent shows, and this has not been contested, that the claimed subject-matter, in particular the method of claim 1, solves this problem.

15. According to the appellant, the method of claim 1 is obvious in the light of a combination of the disclosure in document D9 with one of several other prior art documents, namely documents D8, D7 (an US application corresponding and identical to document D8), D1 and D16. The opposition division, although with regard to claims that were differently worded (cf. points 12 and 13 *supra*), considered that, starting from document D9, there was no incentive for a skilled person to combine this document with any of these prior art documents.

16. Irrespective of the question, whether or not there was a hint in document D9 that would have motivated a skilled person, trying to solve the underlying technical problem, to turn to documents D8/D7, D1 or D16, the appellant has failed to show that any of these documents indeed discloses all technical features which, in combination with the disclosure in document D9, would have led the skilled person in an obvious manner to the method of claim 1, namely to the "*pre-hybridization embodiment*". Upon explicit request of the board, the appellant referred only to these prior art documents in a general manner and to the common general knowledge of the skilled person.

17. The claimed "*pre-hybridization embodiment*" of claim 1 requires the hybridization of a single stranded nucleic acid template to the immobilized first primer species prior to forming the w/o emulsion. This feature provides an advantageous reduction in the number of emulsion droplets devoid of beads with immobilized first primer species and containing only single stranded nucleic acid templates. This improvement is particularly relevant when the amount of single stranded nucleic acid template is scarce or limited, since fewer template is wasted.

18. Thus, the Main Request fulfils the requirements of Article 56 EPC.

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 maintain the patent on the basis of claims 1 - 29 of the Main Request filed at the oral proceedings on 2 March 2016 and a description to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated