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Datasheet for the decision of 24 January 2008

Case Number: T 0768/06 - 3.3.08

Application Number: 96940934.1

Publication Number: 0876509

IPC: C12N 15/10

Language of the proceedings: EN

Title of invention:
Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

Patentee:
Maxygen, Inc.

Opponents:
GENENCOR INTERNATIONAL INC.
Diversa Corporation

Headword:
Molecular evolution/MAXYGEN

Relevant legal provisions:
EPC Art. 56
RPBA Art. 12(2)

Relevant legal provisions (EPC 1973):
EPC Art. 56
RPBA Art. 10a)(2)

Keyword:
"Main request - inventive step - yes"

Decisions cited:
T 0263/05

Catchword:
See Section VI of Facts and Submissions and points 1 to 3 of the Reasons.

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0768/06 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 24 January 2008

Appellant: (Patent Proprietor)	Maxygen, Inc. 515, Galveston Drive Redwood City, CA 94063 (US)
Representative:	Hallybone, Huw George Carpmaels and Ransford 43-45 Bloomsbury Square London WC1A 2RA (GB)
Respondent I: (Opponent 1)	GENENCOR INTERNATIONAL INC., 925 Page Mill Rd. Palo Alto, CA 94304-1013 (US)
Representative	Kiddle, Simon John Mewburn Ellis LLP York House 23 Kingsway London WC2B 6HP (GB)
Respondent II (Opponent 2)	Diversa Corporation 4955 Directors Place San Diego, CA 92121 (US)
Representative:	Dunleavy, Kevin James Knoble & Yoshida LLC p/o De Vries & Metman Overschiestraat 180 NL-1062 XK Amsterdam (NL)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted 16 March 2006 revoking European patent No.0876509 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
C. Rennie-Smith

Summary of Facts and Submissions

I. European patent No. 0 876 509 with the title "Methods for generating polynucleotides having desired characteristics by iterative selection and recombination" was granted with 18 claims based on the International patent application No. PCT/US96/19256 published as WO 97/20078.

Granted claims 1 and 3 read as follows:

"1. A method of evolving a variant polynucleotide for acquisition of a desired functional property, comprising:

conducting a polynucleotide amplification reaction on initial substrates comprising a plurality of variants of a polynucleotide, wherein at least one cycle of the amplification reaction is an incomplete amplification cycle performed under conditions which produce amplification products comprising incompletely extended variants of the polynucleotide, which amplification products are denatured to component strands, which are reannealed in different pairings to form recombined amplification products, which form the substrates for a subsequent cycle of amplification until the amplification products include recombinant variants of the polynucleotide; and selecting or screening the recombinant variants of the polynucleotide to identify at least one recombinant variant having a desired functional property.

3. A method of claim 1 wherein the conditions resulting in incomplete extension are achieved by adding an agent selected from the group consisting of chemical mutagens, intercalating agents, irradiation, polymerases, nucleotide analogs and recA."

Dependent claims 2, 4 to 11 related to further features of the said method.

II. Two oppositions were filed under Article 100(a) to (c) EPC. The opposition division revoked the patent pursuant to Article 101(2) EPC for lack of inventive step.

III. The appellant (patentee) filed a notice of appeal and submitted a statement of grounds of appeal, relying on the claim request filed at oral proceedings on 2 February 2006 and refused by the opposition division. This claim request comprised 11 claims: claims 2, 4 to 11 corresponded to granted claims 2, 4 and 5, 9 to 14; granted claims 6 to 8 were deleted. Claims 1 and 3 read as follows:

"1. A method of evolving a variant polynucleotide for acquisition of a desired functional property, comprising:

conducting a polynucleotide amplification reaction on initial substrates comprising a plurality of variants of a polynucleotide, wherein at least one cycle of the amplification reaction is an incomplete amplification cycle performed under conditions which produce **at least 20%** amplification products comprising incompletely extended variants of the polynucleotide,

which amplification products are denatured to component strands, which are reannealed in different pairings to form recombined amplification products, which form the substrates for a subsequent cycle of amplification until the amplification products include recombinant variants of the polynucleotide **having multiple crossovers**; and selecting or screening the recombinant variants of the polynucleotide to identify at least one recombinant variant having a desired functional property. (differences from granted claim 1 highlighted by the board)

3. A method of claim 1 wherein the conditions resulting in incomplete extension are achieved by adding an additive or polymerase."

IV. In a letter dated 1 November 2006, respondent II (opponent 02) indicated that it did not intend to make written submissions, but intended to rely on those made during opposition proceedings.

V. The board sent summons to oral proceedings to take place on 17 January 2008. It was accompanied by a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal (now Article 15(1) RPBA - cf. OJ EPO 2007, 536) indicating its preliminary, non-binding opinion, wherein inter alia a clarity objection was raised against claim 3.

VI. In a letter of 5 September 2007 the appellant's representative requested postponement of the oral proceedings because he had oral proceedings in another (unrelated) case on the previous day which would make it impossible for him to prepare fully for this case.

In a communication of 18 October 2007 the board refused the request since the reason given was of a personal work pressure nature (see Notice of 16 July 2007 OJ EPO, 2007 Special Edition 3, pages 115-116). The board was not satisfied that the work pressures of one party's representative should outweigh the possible prejudice by delay to the other parties and parties to other appeals whose cases might be delayed by a postponement. Further, there remained ample time to arrange for one of the hearings to be conducted by another representative.

VII. The appellant filed further observations on 17 December 2007, together with an auxiliary request.

VIII. By letters dated 17 and 28 December 2007, respectively, respondent I (opponent 01) and respondent II informed the board that they would not take part in the oral proceedings.

IX. On 4 January 2008, the appellant withdrew its request for oral proceedings conditional on the board allowing its appeal.

X. On 9 January 2008, the board sent a communication to inform all parties that such a conditional request could not be accepted, that the oral proceedings would take place and that, in the absence of the parties, it could be expected that the decision on that date would be to set the opposition division's decision aside and to order the maintenance of the patent on the basis of the main request filed on 2 February 2006.

XI. On 11 January 2008, respondent I withdrew its request for oral proceedings and the appellant withdrew its request for oral proceedings in relation to the main request and requested that the proceedings be continued in writing. The request for oral proceedings was maintained in relation to the auxiliary request.

XII. Oral proceedings were cancelled by fax letter on 14 January 2008.

XIII. The documents mentioned in this decision are the following:

(1): Stemmer, W.P.C., Proc.Natl.Acad.Sci.USA, Vol.91, pages 10747 to 10751, October 1994;

(3): Stemmer, W.P.C., Biotechnology, Vol.13, pages 549 to 552, June 1995;

(6): Meyerhans, A. et al., Nucleic Acids Research, Vol.18, No. 7, pages 1687 to 1691, 1990;

(12): WO 95/22625.

XIV. The appellant's arguments in writing insofar as relevant to the present decision may be summarized as follows:

Main request;
Article 84 EPC; claim 3

There was no clarity problem with this claim. The patent explained that a method in accordance with the

invention could comprise at least one cycle of amplification conducted with an additive under conditions which promoted or enhanced template switching. It, thus, made it clear that an additive could be used to achieve incomplete extension.

Article 56 EPC; claim 1

Documents (1), (3) or (12) had been mentioned as representing the closest prior art. They each disclosed essentially the same "DNA shuffling" method for generating recombinant polynucleotides. It was not important which one was taken as the closest prior art.

The method for generating a pool of different recombinant polynucleotides described in document (1) involved **digesting a large gene into random DNA fragments which were then reassembled into a full length gene** by repeated cycles of annealing in the presence of DNA polymerase. The fragments primed each other and recombination occurred when fragments from one copy of gene primed on another copy, causing a template switch. The end-products of the reaction were screened or selected for having desired functional properties.

The objective problem to be solved by the invention was the provision of an alternative method for evolving variant polynucleotides for acquisition of a desired property.

The solution provided was a method involving multiple cycles of partial extension, denaturation and reannealing of primer polynucleotides in the presence

of template polynucleotides and polymerase enzyme wherein **partially extending primers annealed to the full length polynucleotide template variants.**

The opposition division had concluded that this solution lacked inventive step in view of the teaching of document (1) combined with that of document (6) - which was referred to in document (1) - because the latter described PCR recombinants between two distinct HIV tat gene variants and suggested that recombination between variants arose due to the presence of **incompletely extended primers.** In their opinion, it was thus, obvious to achieve the results described in document (1) by partial extension of primer polynucleotides rather than by full extension of randomly cut fragments.

However, the reference to document (6) in document (1) had been taken out of context. It was not a suggestion for an alternative method of in vitro evolution of polynucleotides but an observation made as part of the author's academic analysis of earlier results of an experiment involving error prone PCR and selection. In fact, document (6) was not in the relevant technical field as it was simply concerned with avoiding the problem of recombination of related sequences during PCR. Taken in its context, the reference to document (6) could only be interpreted as to the production of chimeric molecules for investigating structure/function relationship for viral proteins. Any other interpretation was based on hindsight analysis of the document. In particular, any interpretation in the context of in vitro molecular evolution was completely unjustified. Evidence thereto could be found in the

fact that the document never envisaged the possibility of multiple crossovers and furthermore, contained no suggestion that chimeric molecules might be produced which would benefit from improved properties relative to the starting polynucleotides.

For these reasons, inventive step must be acknowledged.

XV. The appellant requested that the decision under appeal be set aside and that a patent be maintained on the basis of the main request filed on 2 February 2006, alternatively on the basis of the auxiliary request filed on 17 December 2007.

The respondents did not make any requests on appeal.

Reasons for the Decision

The respondents' involvement in the appeal proceedings

1. Respondent II's reply to the appellant's statement of grounds of appeal consisted only of its letter of 1 November 2006 which merely stated:

> "Opponent 02, Diversa Corporation, hereby indicates that at this time it does not intend to make any further written submissions in the above-mentioned appeal proceeding. Diversa Corporation intends to rely on the written submissions made during the opposition proceedings in relation to this European Patent."

Although that letter left open the possibility that respondent II might make later submissions, in fact it played no further part in the proceedings.

2. The board does not consider it necessary to treat respondent II's written submissions in the first instance proceedings as if made anew in the appeal proceedings. Article 12 (formerly Article 10a)2)), paragraph (2) RPBA requires:

> "The statement of grounds of appeal and the reply shall contain a party's complete case. They shall set out clearly and concisely the reasons why it is requested that the decision under appeal be reversed, amended or upheld, and should specify expressly all the facts, arguments and evidence relied on."

The board notes first that respondent II did not even make any request in the appeal proceedings although, since it could be inferred from its two sentence reply that it maintained its opposition to the patent in suit, it could possibly also be inferred that it wanted the appeal to be dismissed or, in the words of Article 12(2) RPBA, that it wanted the decision under appeal to be upheld. More importantly however, the mere cross-reference to written submissions which were made in the opposition proceedings and which, as is clear from the minutes of the oral proceedings before and the decision of the Opposition Division, were not accepted in their entirety, cannot amount to a "complete case" for the request that the decision be upheld and it certainly does not "set out clearly the reasons" for that request and nor does it "specify expressly all the

facts, arguments and evidence relied on". The need for a party in appeal proceedings to make its complete case in express terms in, as the case may be, its statement of grounds of appeal or its reply was (although in a different context) emphasised in T 263/05 (of 28 June 2007, to be published in OJ EPO, see Reasons, paragraphs 7.1 to 7.18, especially 7.11 to 7.14).

3. Since respondent II's reply does not comply with Article 12(2) RPBA, the board is not required to take it into account since Article 12(4) RPBA states:

> "Without prejudice to the power of the Board to hold inadmissible facts, evidence or requests which could have been presented or were not admitted in the first instance proceedings, everything presented by the parties under (1) shall be taken into account by the Board **if and to the extent it relates to the case under appeal and meets the requirements in (2)**." (Emphasis added)

The words emphasised make it a requirement for all written submissions in appeal proceedings that they must comply with Article 12(2) RPBA. Since, as has been shown above, respondent II's reply does not contain a complete case, does not provide reasons for its request (if any), and does not specify expressly all the facts, arguments and evidence relied on, it does not comply with Article 12(2) RPBA and thus does not meet the requirement of Article 12(4) RPBA.

4. Respondent I did not file any reply in answer to the appellant's statement of grounds of appeal and played no other part in the appeal proceedings. It follows

from the Board's observations above about Respondent II that there is no case of Respondent 1 to be considered at all.

Main request
Articles 123(2), 54 and 83 EPC

5. In the absence of any submissions on appeal by the respondents, the board carefully considered the issues of added subject-matter, novelty and sufficiency of disclosure which had been decided in favour of the appellant by the opposition division. In each case, it agrees with the reasoning presented in the decision under appeal and, thus, comes to the conclusion that the requirements of Articles 123(2), 54 and 83 EPC are fulfilled.

Articles 123(3) and 84 EPC
Claim 3

6. Whether the scope of claim 3 exceeded that of granted claim 3 was a matter discussed in the decision of the opposition division. Claim 3 refers to a method of claim 1 wherein the conditions resulting in incomplete extension were said to be achieved by "adding an additive" rather than by adding an additive chosen from a group of specific additives, as in granted claim 3.

7. However, present claim 3 is, like granted claim 3, dependent on claim 1 which in the granted form referred to "conditions which produce amplification products comprising incompletely extended variants", without giving any more information on these conditions and which, thus, covers the use of any additives.

Accordingly, the scope of protection provided by the granted claims remains unchanged (Article 123(3) EPC).

8. In its communication, the board remarked that although the patent undoubtedly disclosed the use of additives, it may not be clear that this use was for the purpose of achieving incomplete extension. In answer, the appellant pointed to paragraph [052] of the granted patent where it was mentioned that the use of an additive would promote or enhance template switching. The board agrees that, on this basis, the skilled person would associate the use of additives with the fact of achieving incomplete extension. The requirements of Article 84 EPC are fulfilled.

Article 56 EPC; inventive step
Claim 1

9. Document (1) describes a method for generating a pool of different recombinant polynucleotides which can be screened or selected for recombinant polynucleotides having desired functional properties. Document (3) is a review of in vitro recombination methods entitled "Searching Sequence Space" which analyses the advantages and disadvantages of the then known methods of recombination and suggests areas of further enquiry. It describes essentially the same method for generating recombinant polynucleotides by DNA shuffling as document (1). Document (12) is the patent publication corresponding to document (1). The three documents provide equivalent teachings and each of them could equally be taken as the closest prior art. The assessment of inventive step will be carried out on the basis of document (1) as closest prior art.

10. Document (1) is entitled "DNA shuffling by random fragmentation and reassembly: *In vitro* recombination for molecular evolution". The method for DNA shuffling is explained in the left-hand column on page 10747 of the document:

> "The method involves digesting a large gene with DNAse I to a pool of random DNA fragments (Fig.2). These fragments can be reassembled into a full-length gene by repeated cycles of annealing in presence of DNA polymerase. The fragments prime each other based on homology, and recombination occurs when fragments from one copy of a gene prime on another copy, causing a template switch."

No full-length templates are present, it is the overlapping single-stranded fragments generated by random cleavage which serve as primers. Because the steps of the method are re-iterated over multiple cycles, the end-products are recombinant polynucleotides having multiple crossovers. These are then screened or selected for desired functional properties.

11. Starting from the closest prior art, the problem to be solved can be defined as the provision of an alternative method of evolving variant polynucleotides for acquisition of a desired functional property.

12. The solution provided is a method whereby the initial variant polynucleotides are not cut into fragments but serve as templates for synthesizing incompletely extended fragments in the polymerase reaction. These

fragments reanneal at random amongst themselves before a further cycle(s) of partial extension is/are carried out. As the partially extended fragments reanneal at random in each cycle, any end-product is likely to be the combination of partially extended fragments from different variant polynucleotides i.e. to be the result of multiple crossovers.

13. Although document (1) (pages 10750 and 10751) discusses various mutagenesis techniques, it contains no suggestion that DNA shuffling itself could be achieved by any other method than the one it describes. Thus, turning, as the opposition division did, to document (6) as a document which, when combined with document (1), would render obvious the present method of DNA shuffling because it taught that recombination occurred during normal PCR, could prima facie be considered as exercising hindsight. However, it is a fact that document (6) is mentioned in document (1) - as bibliographical reference 26 - when discussing earlier methods of protein mutagenesis:

> " Error-prone PCR and oligonucleotide-directed mutagenesis are thus useful for single cycles of fine tuning but rapidly become limiting when applied to multiple cycles... Using the Lac assay (Fig.3), recombination has been found to occur even during normal PCR at a frequency of about 0.03% over 25 cycles. Others have reported a rate of 5.4% recombinants under standard PCR conditions (26)..."

14. It may be expected that the skilled person would read any documents cited in the closest prior art for the

simple reason that he/she would wish to have as extensive a knowledge as possible of how this prior art came to be. Thus, he/she would learn from document (6) that there existed a possibility of recombination at specific sites on two variants of the HIV1 tat gene being co-amplified in the same PCR. Furthermore, he/she would become aware of the suggestion that the phenomenon could be exploited to create chimeric molecules from related sequences (abstract of document (6)). The question is: does this suggestion - in combination with the teachings of document (1) - make it obvious to engineer molecular evolution as is done by the instant invention ?

15. In the board's judgement, the question must be answered in the negative. Document (6) refers to the production of chimeric molecules in the framework of obtaining chimeric proteins for investigating structure/function relationship. This project is not aimed at all at creating molecular diversity on the scale required for molecular evolution. This is readily evident from the document itself where it is not envisaged to generate recombinant polynucleotides having multiple crossovers. Thus, in order to solve the above mentioned problem, the present inventors had to devise a method which is conceptually different from that described in document (1) which entailed exploiting the mechanism of recombination over numerous cycles, a course of action which had never been envisaged in document (6). For these reasons, inventive step is acknowledged.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is sent back to the first instance with the order to maintain the patent on the basis of the following documents:

   - claims 1 to 11 filed on 2 February 2006, and

   - a description to be adapted thereto and

   - the Figures 1 to 33b as granted.

The Registrar The Chairman

A. Wolinski L. Galligani