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Datasheet for the decision of 12 December 2007

Case Number: T 0582/06 - 3.3.08

Application Number: 95911826.6

Publication Number: 0752008

IPC: C12N 15/10

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Title of invention:
DNA mutagenesis by random fragmentation and reassembly

Patentee:
Maxygen, Inc.

Opponents:
GENENCOR INTERNATIONAL INC.,
Diversa Corporation
Koninklijke DSM N.V.
Alligator Bioscience AB

Headword:
DNA mutagenesis/MAXYGEN

Relevant legal provisions (EPC 1973):
EPC Art. 123, 54, 56, 83

Keyword:
"Main request - added subject-matter - yes"
"Auxiliary request - added subject-matter - no"
"Novelty - Inventive step - Sufficiency of disclosure - yes"

Decisions cited:
T 0606/89, T 0520/01

Catchword:
-

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Case Number: T 0582/06 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 12 December 2007

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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 21 February 2006 concerning maintenance of European patent No. 0752008 in amended form.

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
T. Karamanli

Summary of Facts and Submissions

I. European patent No. 0 752 008 with the title "DNA mutagenesis by random fragmentation and reassembly." was granted with 32 claims based on the International Application No. PCT/US95/02126 published as WO 95/22625.

Granted claims 1, 10 and 31 read as follows:

"1. A method for molecular evolution of a template polynucleotide into a mutagenized double-stranded polynucleotide comprising:

a) providing a population of double-stranded overlapping fragments of the template double-stranded polynucleotide and one or more single or double-stranded oligonucleotides, wherein said oligonucleotides comprise an area of homology and an area of heterology to the template double-stranded polynucleotide;

b) denaturing the resultant mixture of double-stranded overlapping fragments and oligonucleotides into single-stranded fragments;

c) incubating the resultant population of single-stranded fragments with a polymerase under conditions which result in the annealing of said single-stranded fragments at said areas of homology to form pairs of annealed fragments, said areas of homology being sufficient for one member of a pair to prime replication of the other thereby forming mutagenized double-stranded polynucleotides; and

d) repeating steps (b) and (c) for at least two further cycles, wherein the resultant mixture in step (b) of a further cycle includes the mutagenized double-stranded polynucleotides from step (c) of the previous cycle, and the further cycle forms further mutagenized double-stranded polynucleotides; and

e) selecting or screening further mutagenized polynucleotides to identify an evolved form of the template polynucleotide with a desired functional property.

10. A method for obtaining a chimeric polynucleotide sequence by molecular evolution of sequence-related template polynucleotides comprising:

a) fragmenting different double-stranded template polynucleotides in a sample wherein said different template polynucleotides contain areas of homology and areas of heterology under conditions whereby overlapping double-stranded fragments of a desired size of said different double-stranded template polynucleotides are formed;

b) denaturing the relevant overlapping double-stranded fragments of said different double-stranded template polynucleotides contained in the treated sample produced by step (a) into single-stranded fragments;

c) incubating the resultant single-stranded fragments with polymerase under conditions which provide for the annealing of the single-stranded fragments at the areas of homology to form pairs of

annealed fragments, said areas of homology being sufficient for one member of a pair to prime replication of the other thereby forming chimeric double-stranded polynucleotide sequences comprising template polynucleotide sequences; and

d) repeating steps (b) and (c) for at least two cycles, wherein the resultant mixture in step (b) of a cycle includes the chimeric double-stranded polynucleotide sequences in step (c), and the further cycle forms further chimeric polynucleotide sequences; and

e) screening or selecting further chimeric polynucleotide sequences to identify at least one chimeric polynucleotide sequence that is an evolved form of a template polynucleotide with a desired functional property.

31. Use of multiple cycles of denaturation, renaturation and incubation in the presence of a polymerase to shuffle polynucleotide variants whereby overlapping segments having sequences from the variants denature and reanneal in new combinations in which the segments prime each other to form recombinant polynucleotides, whereby the recombinant polynucleotides or their expression products are screened or selected for a preselected functional property."

Dependent claims 2 to 9 related to further features of the method of claim 1. Dependent claims 11 to 18 related to further features of claim 10. Claim 19 was directed to a method for generating a library of

displayed peptides or displayed antibodies suitable for affinity interaction screening or phenotypic screening and dependent claims 20 to 28 related to further features of said method. Claim 29 was directed to a method of constructing a double-stranded polynucleotide *in vitro* from a starting population of nucleic acids comprising double-stranded overlapping nucleic acids and dependent claim 30 related to a further feature of said method. Claim 32 related to a further feature of the methods and use of any of the preceding claims.

II. Three oppositions were filed on the grounds of opposition under Article 100(a) to (c) EPC as well as a notice of intervention pursuant to Article 105 EPC. The opposition division maintained the patent in amended form pursuant to Article 102(3) EPC on the basis of the third auxiliary request then on file.

III. The intervener-opponent 04 (appellant I) as well as the patentee (appellant II) filed notices of appeal on 10 and 19 April 2006, respectively, and they submitted statements of grounds of appeal on 20 and 28 June 2006, respectively. Appellant's II statement of grounds of appeal was accompanied by a main request (claims 1 to 27 filed on 31 January 2005 and refused by the opposition division) and six auxiliary requests.

Claim 27 of the **main request** read as follows:

"27. Use of multiple cycles of denaturation, renaturation and incubation in the presence of a polymerase to shuffle polynucleotide variants whereby overlapping **fragments of the variants produced by random cleavage** denature and reanneal in new

combinations in which the **fragments** prime each other to form recombinant polynucleotides, whereby the recombinant polynucleotides or their expression products are screened or selected for a preselected functional property." (differences from granted claim 31 emphasized by the board).

Claims 1 and 9 respectively related to "a method for introducing one or more mutations into a template double-stranded polynucleotide ..." and to "a method for obtaining a chimeric polynucleotide..." each comprising the same methods steps a) to e) as granted claims 1 and 9.

IV. By a letter dated 1 November 2006, opponent 02 (respondent II) indicated that it did not intend to make written submissions. On 20 November 2006, appellants I and II filed further submissions in answer to their respective statements of grounds of appeal.

V. The board sent a summons to oral proceedings to take place on 12 December 2007. They were accompanied by a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal, indicating its preliminary, non-binding opinion.

VI. Appellant I filed further observations. By letters dated 10 and 11 December 2007, opponents 01 and 03 (respondents I and III) informed the board that they would not take part in the oral proceedings.

VII. During oral proceedings, appellant II filed a new auxiliary request 1 comprising claims 1 to 27 in replacement of auxiliary request 1 filed with the

statement of grounds of appeal. Claims 1 to 25 and 27 of this new auxiliary request were identical to claims 1 to 11, 13 to 26 and 28 of the main request.

Claim 26 of this new **auxiliary** request 1 read as follows:

"26. Use of multiple cycles of denaturation, renaturation and incubation in the presence of a polymerase to shuffle **template double-stranded** polynucleotide variants whereby random overlapping fragments of the variants produced by random cleavage denature and reanneal in new combinations in which the fragments prime each other to form recombinant polynucleotides, whereby the recombinant polynucleotides or their expression products are screened or selected for a preselected functional property."(differences from claim 27 of the main request emphasized by the board).

VIII. The following documents are mentioned in this decision:

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

(6): Pääbo, S. et al., The Journal of Biological Chemistry, Vol.265, No.8, 15 March 1990, pages 4718 to 4721;

(7): Marton, A. et al., Nucleic Acids Research, Vol.19, No.9, 1991, pages 2423 to 2426;

(29): Balint, R.F. and Larrick, J.W., Gene, Vol.137, 1993, pages 109 to 118;

(31): Horton, R.M. and Pease, L.R., in "Directed Mutagenesis; A Practical Approach", Chapter 11, paragraph 6.1.3, page 243, 1991, edited by M.J. McPherson, IRL Press Oxford UK.

IX. Appellant I's arguments filed in writing and submitted during oral proceedings insofar as relevant to the present decision may be summarized as follows:

Main request
Article 123(2)(3) EPC

- Claims 1 and 9: these claims differed from granted claims 1 and 10 which had been limited by reference to the "molecular evolution" of a double-stranded template polynucleotide (see sections I and III). When required by the examining division to delete this feature, the patentee had declined to do so. This should be regarded as an implicit acknowledgment that the feature was not purely cosmetic. Accordingly, its deletion from present claims 1 and 9 amounted to a broadening of the scope of the claims (Article 123(3) EPC).

- Claim 27:
"shuffling of polynucleotide **variants**"
Claim 27 corresponded to granted claim 31 which found no counterpart in the application as filed. The claimed subject-matter encompassed the use of multiple cycles of denaturation, renaturation ... to shuffle **single-stranded** as well as double-stranded polynucleotide

variants. Yet, there was no disclosure of shuffling single-stranded variants in the application as filed. On the contrary, the double-stranded nature of the template and its fragments was presented as an essential feature of the invention, e.g. on page 24, line 38 to page 25, line 4 of the application as filed (published form). The fact that the fragments themselves must be double-stranded was confirmed by the patentee itself in its letter of 24 November 2003. As for the passage on page 29, line 34 to page 30, line 2 of the application as filed which mentioned single-stranded DNA, it was not referring to the nature of the template but to that of any starting nucleic acid.

"**shuffling** of polynucleotide variants"
In the application as filed (passage bridging pages 12 and 13), the term "DNA shuffling" was said to be used "to indicate recombination between substantially homologous but non-identical sequences". The term "variants" covered more molecules than the term "substantially homologous sequences". It inevitably followed that by relating to the shuffling of polynucleotide variants, claim 27 comprised subject-matter which was not disclosed in the application as filed.

If only for these reasons, claim 27 did not fulfil the requirements of Article 123(2) EPC.

- Claims 1, 9 and 27:
"overlapping fragments priming each other"
The features of producing **overlapping** fragments of the variants had not been disclosed in the application as filed which simply referred to random fragments of a desired size (e.g. page 8, line 21 to page 9, line 4). There was also no basis in the application as filed for the teaching that the fragments would **prime each other**.

Auxiliary request 1 filed at oral proceedings
Article 123(2),(3) EPC

- The objections raised against claims 1, 9 (Article 123(3) EPC) and claims 1, 9 and 27 (Article 123(2) EPC) of the main request (see supra) equally applied to claims 1, 9 and 26 of this request.

- Claim 26 still encompassed the possibility that overlapping fragments of the template double-stranded polynucleotide variants be in single-stranded form, a feature which was not disclosed in the application as filed.

For these reasons, none of claims 1, 9 and 26 were allowable under Article 123(2),(3) EPC.

Article 54 EPC

Even if the claimed subject-matter was considered to enjoy priority of the priority application US 08/198 431 with the filing date of 17 February 1994, it remained that claims 1, 9 and 26 lacked novelty over the disclosures of document (6) or (7).

Document (6) described a study demonstrating how DNA damage promoted jumping of extending strands between templates during PCR, which resulted in the formation of recombined products. Template polynucleotides were randomly cleaved to generate overlapping fragments which underwent a PCR-like reaction in the presence of oligonucleotide primers. Since full-length recombined products were generated, it was implicit that the random fragments had been priming each other. The further sequencing of the "reconstituted" polynucleotides constituted a screening for a functional property of the DNA.
Document (7) described another study demonstrating that nicking or breaking led to the formation of chimeric recombined PCR products. The experimental protocol used comprised all of the features of the methods of claims 1, 9 or 26. As it is the case with document (6), the sequencing of the final products constituted screening for a functional property.

Both documents, thus, taught methods comprising the same steps as the claimed methods and were detrimental to novelty.

Article 56 EPC
Claims 9 and 26

- Document (31), a textbook entitled "Directed mutagenesis", could be regarded as the closest prior art. Recombination and mutagenesis of DNA sequences using PCR was discussed in the chapter 11, paragraph 6, on page 243.
Starting from this document, the objective problem to be solved could be defined as implementing the

suggestion contained in paragraph 6 to utilise random recombination between related genes being amplified in the same PCR to generate sets of random recombinants.
A possible difference between the disclosure in document (31) and the subject-matter of claim 9 could be seen in the selection of a method of damaging the template. In this respect, document (6) - relating to how DNA damage promotes jumping between templates - would certainly have been considered by the skilled person, as the phenomena underlying jumping PCR and the method of the alleged invention were, in fact, identical. Document (6) (page 1720) disclosed that random damaging to the template could be achieved by sonication, depurination or UV irradiation, all such methods being equally contemplated in the patent in suit ([0153] of the patent specification]).
For these reasons, the combination of the teachings of documents (31) and (6) was detrimental to inventive step.

- Alternatively, document (5) could also be considered as the closest prior art because it taught that during PCR co-amplification of different polynucleotide sequences, random crossover or recombination events led to the generation of chimeric polynucleotides.
Starting from document (5), a skilled person would look at document (7) when seeking to implement the suggestion to use PCR recombination to produce chimeric molecules. Their combined teachings would have made it obvious that breaking or nicking the DNA template by random cleavage would be a method for optimizing the conditions for the generation of chimeric molecules.

The requirement of Article 56 EPC was not fulfilled.

X. Appellant II's arguments filed in writing and submitted during oral proceedings insofar as relevant to the present decision may be summarized as follows:

Main request
Article 123(2)(3) EPC

- Many of the arguments raised in appellant I's statement of grounds of appeal had not been discussed at oral proceedings before the opposition division because they had already been dealt with by the patentee's written submissions and so, they did not form part of the decision of the opposition division. The finality of the appeal proceedings was to provide an opportunity to review this decision. Accordingly, appellant I's arguments should be disregarded. This course of action was fully supported by the case law (e.g. T 520/01 of 29 October 2003).

- Claims 1 and 9
The absence of the term "molecular evolution" from the wording of these claims did not amount to an extension of the scope of protection provided by the patent because the term was not a significant technical limitation but rather a convenient descriptor of what the claimed methodology set out to achieve. The requirements of Article 123(3) EPC were fulfilled.

- Claim 27
"shuffling of polynucleotide **variants**"
The subject-matter of this claim was a method for shuffling DNA, its essential features being the method steps themselves: denaturation, renaturation,

incubation, priming... This was reflected in the fact that the claim specified neither the physical nature of the starting material nor that of the template or fragments of the template.
The skilled person would derive from the application as filed taken as a whole that the structure of the DNA variants was irrelevant for carrying out the claimed method. Various passages (e.g. page 23, lines 9 to 14, page 27, line 35 to page 28, line 2, page 5, line 36 to page 6, line 1) clearly taught the skilled reader that the claimed use could be carried out on any DNA template, irrespective of it being double or single-stranded.
Appellant I had misrepresented the statement on page 24, line 38 to page 25, line 4 as necessarily implying that the DNA must be double-stranded. In fact, what was mentioned there, was that "a template polynucleotide often should be double-stranded". Admittedly, the combination of "often" and "should" was somewhat unusual English. Yet, "often" left no doubt that double-strandedness was not a mandatory feature of the template structure. Furthermore, such passages in the application as filed cited by Appellant I which referred to double-stranded DNA were only representative of specific ways of carrying out the invention.
For these reasons, the claimed use which comprised shuffling single- as well as double-stranded DNA variants did not offend Article 123(2) EPC.

"**shuffling** of polynucleotide variants"
Appellant I had argued that this expression went beyond the definition of the term "shuffling" in the application as filed as "to indicate recombination

between substantially homologous ... sequences", because no degree of homology could be attached to the term "variants". This argument was not valid, firstly, because the passage did not provide a definition of the variants but was simply a mention of what happened during recombination. Secondly, when the specification as filed was considered as a whole (e.g. page 9, lines 17 to 31, page 23, lines 9 to 14 ...), it clearly disclosed that the fragments should be similar enough to prime off each other, yet different enough for recombinant polynucleotides to be formed.

None of these objections under Article 123(2) EPC against claim 27 were convincing.

- Claims 1, 9 and 27:
"overlapping fragments priming each other"
It was true that the term "overlapping fragments" did not appear in the application as filed. Yet, random fragmentation of the different template polynucleotides was undoubtedly disclosed, which necessarily resulted in a population of overlapping fragments.
In the same manner, the phenomenon of fragments priming each other was inherent in the process of denaturation renaturation and incubation of randomly fragmented templates in the presence of a polymerase. Accordingly, reference to overlapping fragments priming each other in the claims did not contravene Article 123(2) EPC.

Auxiliary request 1 filed at oral proceedings
Article 123(2,(3) EPC

- The reasons why claims 1, 9 (Article 123(3) EPC) and claims 1, 9 and 27 (Article 123(2) EPC) of the main

request did not go beyond the scope of the granted claims nor contained added subject-matter remained valid for claims 1, 9 and 26 of this request.

- The limitation of claim 26 to the shuffling of "template **double-stranded** polynucleotide variants whereby random overlapping fragments of the variants produced by random cleavage denature ..." made it unambiguous that the template and fragments thereof had to be double-stranded. These features were disclosed throughout the application as filed.

Article 54 EPC

Claims 1, 9 and 26 were novel over the teachings of documents (6) or (7) if only because the experiments described in either of these documents did not comprise the step of selecting/screening further mutagenized or chimeric polynucleotides (claims 1 and 9)/recombinant polynucleotides or their expression products (claim 26) for a desired/preselected functional property.
In fact, these documents were research papers on the extent to which recombination occurred during co-amplification of DNAs and the end-products of the PCR reactions were sequenced or otherwise identified as recombinants. No steps had been taken to ensure that the sequence of the recombinant polynucleotides or their expression products - which in any case were not obtained - would exhibit a defined/preselected functional property. Indeed, the authors had simply been concerned with observing a recombination event.

Article 56 EPC

- Document (31) was a collection of research papers on Directed Mutagenesis". Section 6.1.3 of Chapter 11 was only a very small part of the overall content of that chapter, and an even smaller part of the overall content of the document itself. It was completely unrealistic for that tiny speculative section to be selected as the closest prior art.

- The closest prior art was document (29) which was in the same technical field as the invention since it described a method for the in vitro molecular evolution of proteins.

The claimed subject-matter provided alternative methods for generating variant polynucleotides.

The difference between these methods and the former one was the production of populations of variant polynucleotides with functionally significant diversity whereas in the populations of variant polynucleotides as produced in the art, almost all of the diversity generated was functionally useless.

The claimed method/use was inventive over the combination of the teachings of document (29) and the prior art documents. In particular, the skilled person starting from document (29) would not have looked to documents (5), (7) or (31) which were not in the field of molecular evolution. In addition, none of these documents came anywhere to suggesting that random cleavage of template polynucleotides to form random overlapping fragments followed by a polymerase

catalyzed inverse chain reaction might provide variant polynucleotides containing significant diversity. Yet, if polynucleotides do not contain significant functional diversity, they will not be useful for in vitro evolution. Only after the invention had been made, did it become evident that a library of recombinant sequences containing functionally significant diversity could be recovered from a pool of randomly fragmented nucleotide sequences.

For these reasons, the claimed subject-matter was inventive.

XI. Appellant I (opponent 04) requested that the decision under appeal be set aside and the patent be revoked.

Appellant II (patentee) requested that the decision under appeal be set aside and that the patent be maintained in amended form on the basis of the main request filed with letter of 31 January 2005 or, in the alternative, on the basis of auxiliary request 1 filed during the oral proceedings.

Reasons for the Decision:

Article 123(2) EPC

1. In the decision under appeal, the opposition division refused the main request because, in their view, some of the claims (e.g. claim 27) did not enjoy priority rights from the priority application US 08/198 431 and, thus, lacked novelty over the teachings of documents published in the priority interval. Prior to

considering these issues, the opposition division had carried out an assessment of the claims under Article 123(2) EPC which had voluntarily been restricted "in view of the decision on priority and novelty" which followed. Thus, most of the objections raised under Article 123(2),(3) EPC by the opponents were not discussed. This, however, does not in any way mean that they have ceased to exist or are of no relevance. Pursuant to Article 111(1) EPC, the board will exercise the competence of the opposition division in this respect and full compliance with Article 123(2) EPC will be one of the issues dealt with in this decision.

2. Appellant II made reference to the case law (T 520/01, supra) to back up its position that arguments under Article 123(2) EPC other than the one reviewed by the opposition division should not be considered because the purpose of the appeal was solely to revise the decision of the first instance. Yet, T 520/01 establishes that in a case where a **ground** of opposition was expressly not maintained in opposition oral proceedings and the opposition division did not deal with it in their decision, the re-introduction of this ground in appeal proceedings constituted a fresh ground which could only be taken up with the proprietor's permission. These findings are not relevant to the present case insofar as the ground of added subject-matter (Article 123(2) EPC) was never abandoned.

Main request; claim 27
Article 123(2) EPC

3. Claim 27 corresponds to granted claim 31 (see sections I and II, supra). The claimed use requires that a number of steps be carried out on polynucleotide variants, comprising the production of overlapping fragments which are handled further until molecular diversity is achieved. Some molecules with preselected functional properties are formed which may be screened or selected for. The absence of any characterising features of the DNA substrates (variants/fragments) leaves it open that they may be single- or double-stranded. In this respect, the board accepts appellant II's arguments that conceptually, it is the series of steps leading to DNA shuffling which are important. Nonetheless, it remains that, like any others, the corresponding invention relates to a technical achievement and, that, in order that the requirements of Article 123(2) EPC be fulfilled, the technical achievement - including the nature of the substrates of the claimed use - must be clearly and unambiguously disclosed in the application as filed.

4. The question to be settled is, thus, whether or not the application as filed discloses in a clear and unambiguous manner that the claimed use may be carried out on single-stranded as well as double-stranded template polynucleotides.

5. In this respect, appellant II pointed out to various passages in the application as filed:
Page 5, line 36 to page 6, line 1: "The present invention is directed to a method for generating a

selected polynucleotide sequence or population of selected polynucleotide sequences, typically in the form of amplified and/or cloned polynucleotides, whereby the selected polynucleotide sequence(s) possess a desired phenotypic characteristic .... which can be selected for."
Page 23, lines 9 to 11: "Nucleic acid shuffling is a method for *in vitro* or *in vivo* homologous recombination of pools of nucleic acid fragments or polynucleotides."
Page 27, lines 35 to 38: "The cycle of denaturation, renaturation and incubation in the presence of a polymerase is refered to herein as shuffling or reassembly of the nucleic acid. This cycle is repeated for a desired number of times".

6. In the board's judgement, the first two passages describe the aim of the method and not the way(s) to perform it. On the contrary, the third passage discloses the steps involved, yet it is wholly silent as to the substrate(s) on which to carry it out. It is accepted that at some point in the cycle, the DNA will be in single-stranded form whereas at others, it will be double-stranded. This is not, however, an equivalent teaching to that which would identify the nature of the polynucleotide variants - the starting point of going into the cycle.

7. There is also the last sentence on page 24 of the application as filed containing the statement that: "The template polynucleotide **often should be** double-stranded." (emphasis added by the board). In the board's judgement, this wording is confusing. The sentence could be understood as meaning that the template polynucleotide need not be double-stranded

because of the word "often". However, in the board's judgement, juxtaposition of this word with the verb "should" which is to be understood as "must", renders moot this interpretation. This view is clearly reinforced by reading the next sentence of the same paragraph on page 25: "A double-stranded nucleic acid molecule is required to ensure that regions of the resulting single-stranded nucleic acid fragments are complementary to each other." The passage as a whole certainly does not amount to a clear and unambiguous disclosure of a single-stranded DNA template.

8. When the nature of the template polynucleotide is mentioned in the application as filed, it is as being double-stranded (e.g. page 8, line 21 to page 9, line 31 and examples). Appellant II argued that it was only defined as such when describing specific embodiments. Irrespective of whether this is true or not, it is reasonable to understand the specific embodiments as illustrating the invention. They cannot but lead to the conclusion that the claimed use is intended with double-stranded polynucleotide templates.

9. For these reasons, the board concludes that claim 27 comprises subject-matter which is not disclosed in the application as filed and, thus, does not fulfil the requirements of Article 123(2) EPC.

Auxiliary request 1
Claims 1 and 9
Article 123(3) EPC

10. These claims are identical to claims 1 and 9 of the main request and they differ from granted claims 1 and 10 in that the claimed methods are not defined as "for molecular evolution of a template polynucleotide ..." (granted claim 1) or as "for obtaining a chimeric polynucleotide sequence by molecular evolution..." (granted claim 10) but as "for introducing one or more mutations into a template double-stranded polynucleotide..." (claim 1) or as "for obtaining a chimeric polynucleotide..." (claim 9), (see sections I and III, supra). The characterising parts of the claims (steps a) to e)) remain identical.

11. On page 4, paragraph [0024] of the granted patent, it is taught that: "The invention described therein is directed to the use of repeated cycles of point mutagenesis, nucleic acid shuffling and selection which allow for the direct molecular evolution *in vitro* of highly complex linear sequences, such as proteins through random recombination." Thus, in the board's judgement, the term "molecular evolution" does not bring any information in addition to that of having to perform steps a) to e). As argued by appellant II, it is merely a convenient way of describing what the claimed methods set out to achieve. The scope of the claims is not extended by omitting this term. The requirements of Article 123(3) EPC are fulfilled.

Claim 26
Article 123(2) EPC

12. This claim - corresponding to claim 27 of the main request - is restricted to the shuffling of **double-stranded** polynucleotide variants. At oral proceedings, it was not disputed that the application as filed described the template polynucleotide as being double-stranded. Rather, the argument went to the point that the physical structure of the overlapping fragments to be derived therefrom by random cleavage had not been specified in the claim.

13. For the board, the only interpretation to be given to this claim is that it is directed to a method of shuffling whereby the template variant polynucleotides **and** the overlapping fragments produced by random cleavage are **double-stranded.** This is unambiguously derivable from the fact that the overlapping fragments are said to be of the template double-stranded variants **and** from the fact that the overlapping fragments are said to denature. Were they not double-stranded, they could not denature.

14. The argument that the claim still covered the possibility that the overlapping fragments be single-stranded - while the template was double-stranded - was presented for the reason that the term "denature" would reflect the heating of single- stranded fragments for destroying any secondary structure which may have formed within the single-strand prior to the polymerisation step. This argument is not found convincing insofar as the denaturation is to take place on fragments of variants which are identified as

double-stranded. Accordingly, the only meaning which can be given to the term "denature" is that the denaturation affects double-stranded DNA.

15. The application as filed teaches a double-stranded DNA template being randomly cleaved into double stranded fragments (e.g. page 8, line 21 to page 9, line 31) and, therefore, the claimed feature does not offend the requirements of Article 123(2) EPC. Further objections under this Article were raised against claim 26 which also applied to claims 1 and 9. They will be considered below.

Claims 1, 9 and 26;
"overlapping fragments priming each other"; "shuffling of polynucleotide variants"
Article 123(2) EPC

16. It is true that the application as filed does not mention expressis verbis "overlapping fragments priming each other" but it discloses "random fragments of a desired size" (page 8, line 21 to page 9, line 31). The fact that the template is double-stranded necessarily implies that its random cleavage will result in overlapping double-stranded fragments being produced. Being overlapping, these fragments will have some sequences in common and, therefore, will be capable of priming each other after denaturation.

17. The expression "**shuffling** of polynucleotide variants" does not appear in the application as filed. In the passage bridging pages 12 and 13, the term "shuffling" is said "to indicate recombination between substantially homologous but non-identical

sequences...". Appellant I argued that the term "polynucleotide variants" did not necessarily carry forward the meaning that the variants must be substantially homologous and that, therefore, the claimed subject-matter went beyond that disclosed in the application as filed. In the board's judgement, the above mentioned definition of the term "shuffling" simply discloses that recombination takes place in substantially homologous regions. This is not to say that the variants have to be substantially homologous. The general teaching in the application (e.g. page 9, lines 17 to 31, page 23, lines 9 to 14) leaves no doubt that the variants must be sufficiently homologous in some regions for recombination to occur.

18. For the reasons explained in points 10 to 17, supra, the claims of the first auxiliary request fulfil the requirements of Article 123(2),(3) EPC.

Claims 1, 9 and 26
Articles 87 and 88 EPC; Article 54 EPC

19. It was not challenged that the priority application US 08/198 431 disclosed the claimed methods/use in relation to double-stranded polynucleotide variants involving the production of double-stranded overlapping fragments. The passage on page 5, line 4 to page 6, line 26 of the priority document is identical to the passage on page 5, line 44 to page 6, line 12 of the specification of the granted patent. It describes methods in accordance with the invention corresponding to the methods of claim 1, 9 and to the claimed use. Examples 1 to 7 are also present in the patent in suit and in the priority document. Thus, right to priority

is acknowledged and only those documents published before the filing date of the priority application (17 February 1994) need be taken into account for the assessment of novelty.

20. Documents (6) or (7) are research articles published before that date and aimed at finding out whether or not and to which extent DNA damage increases recombination during PCR. At oral proceedings, both were argued to disclose methods comprising the same steps as the method/use of claims 9 and 26. Rather than going into details as to the identity/lack thereof of these steps with steps a) to d), it is expedient to focus on the last step (e): that of " screening or selecting further chimeric polynucleotides to identify at least one chimeric polynucleotide with a desired functional property" (claim 9) and "whereby the recombinant polynucleotides or their expression products are screened or selected for a preselected functional property" (claim 26), and to assess whether such a course of action is envisaged in either of the prior art documents.

21. In the framework of establishing the mechanisms possibly occurring when PCR is carried out "under conditions of DNA damage", the end-products of the PCR reaction are either sequenced (document (6), page 4719, right-hand column, middle par.) or screened by hybridisation to a radioactive probe for having acquired a recombined structure (document (7), page 2424, right-hand column, second paragraph). It is found that at least some of them are the result of recombination. However, at no time is an attempt made to screen or select for a combination of mutations (due

to recombination) resulting in a desired or preselected functional property.

22. Appellant I argued that the identification of some of the end-products as being recombined corresponded to the screening of a desired or preselected functional property, in that it gave evidence of a recombined sequence which had been looked for at the beginning of the study. The board does not agree. The fact that the sequence of the end-product is the result of the recombination event does not mean that this specific sequence had been **preselected**. In fact, the authors simply observed what was given to them by the experiment, they were carrying out. Let alone does it make this sequence a sequence with desired/preselected **functional** property as there is no evidence given of its function and not all variations in sequence would necessarily lead to a functional change.

23. For these reasons at least, the subject-matter of claims 9 and 26 is novel. The same is true of the subject-matter of claim 1, the novelty of which had been challenged during the written proceedings and so for the same reason, as claim 1 contains a step e) equivalent to step e) of claims 9 and 26. Novelty is acknowledged.

Article 56 EPC
Claim 26

24. Documents (29) and (31) were argued by, respectively, appellants II and I to be the closest prior art. According to appellant I, document (5) was also a possible starting point for assessing inventive step.

25. Document (29) is concerned with obtaining without immunisation human antibodies with such an affinity for antigens as is generally required for therapeutic use. The authors, thus, developed a computer-assisted method for oligodeoxyribonucleotide-directed scanning mutagenesis called "parsimonious mutagenesis". This method is used for searching and identifying improved variants in all three complementarity-determining (CDR) regions of the variable region (V-region) of the antibody gene. A library of variants randomly mutated in the CDRs is constructed which is used for probing the antigen surface. For the actual construction of the library, a gene cassette encoding an entire V-region with three randomized CDR is assembled from two overlapping duplexes which are amplified by PCR from the parent sequence using primers containing the randomized CDR. The final assembly step is accomplished by re-amplification of both fragments in the same tube with primers complementary to the V-region ends. When inserted in appropriate constructs such libraries can be expressed as single chain Fv or Fab fragments on phage which can be panned for target (antigen) binding affinity or target rate constant. Document (29) thus teaches a generic method for creating molecular diversity in the V-region and for engineering molecular evolution by screening for variants of this region with the desired functional property of exhibiting a high affinity for antigens.

26. Document (5) is a research article on the events which may occur during PCR co-amplification of two distinct but related DNA sequences (HIV1 tat gene). It is observed that recombination between the sequences

occurs in discrete regions of the DNA where the Taq polymerase may pause or terminate. This recombination is seen as a disadvantage eg when studying individual members of a multigene family. It is noted, however, that the phenomenon can be exploited "to produce a series of chimeric molecules in a single experiment" (page 1690, right-hand column). There is no suggestion in the article that the observed phenomenon could be put to use to create molecular diversity on a scale suitable for achieving *in vitro* molecular evolution.

27. Document (31) is a book entitled "Directed Mutagenesis, A Practical Approach" which comprises 11 chapters covering all methods known in 1991 for the introduction of mutations into DNA. At the end of the last chapter concerned with recombination and mutagenesis of DNA sequences using PCR, there exists a 14 lines long sub-paragraph (par.6.1.3, "random recombination") where it is mentioned in particular that recombination between related genes being amplified in the same PCR reaction may provide a convenient way to generate sets of random recombinants between homologous genes. Furthermore, the indication is given that, in order to increase recombination frequency, it might be useful to damage the template DNA first. This teaching does not significantly differ from that of document (5). In particular, there is no suggestion that the observed phenomenon of recombination could be exploited on such a scale as to achieve molecular evolution.

28. In accordance with the case law (e.g. T 606/89 of 18 September 1990), the closest prior art for assessing inventive step is a prior art document disclosing subject-matter conceived for the same purpose or aiming

at the same objective as the claimed invention and having the most relevant technical features in common.

29. The summaries above leave no doubt that document (29) is the only one to disclose a method for the same purpose as the presently claimed methods and, therefore, it is the closest prior art.

30. Starting from the closest prior art, the problem to be solved may be defined as providing an alternative method to engineer protein diversity.

31. The solution provided is that random overlapping fragments of a population of variant polynucleotides are made to denature, reanneal amongst themselves and extend from each other, these steps being repeated over a few cycles, so that multiple random mutations be "re-grouped" in the same molecule. This solution constitutes a totally different approach to engineering molecular diversity from that used in document (29) which required that molecular diversity be generated during PCR by the use of randomized primers.

32. The claimed use was developed starting from the scientific observation in document (5) - and also in document (7), see points 20 and 21, supra - that in anyone cycle of the PCR reaction, accidentally partially extended fragments may act as primers for further elongation leading to the formation of recombinant chimeric molecules as "artefacts of the extension by polymerase". Document (31) adds to this teaching that the frequency of artefacts may be enhanced by damaging the template thus leading to a higher frequency of random recombination, i.e. chimeric

molecules. As already mentioned in points 26 and 27 supra, none of the documents however, contemplate that generic molecular diversity could be engineered on this basis.

33. In fact, the now claimed method of DNA shuffling is far from a simple and straightforward "put to use" of the phenomena described in documents (5) or (7), even as envisaged in document (31). It is not a PCR reaction because no exogenous flanking primers are added and so there is no exponential amplification of the template. The "artefact" is "translated" into an essential method step by ensuring that the substrates of the polymerase reaction are random overlapping molecules. In addition, the further step of isolating from the population of multiply mutated end-products only those which are regarded as of interest is included. None of these steps are obvious from the prior art.

34. For these reasons, inventive step is acknowledged for the subject-matter of claim 26. The further independent claims 1, 9 and 16 (corresponding to granted claim 19, section I supra) all contain the same method steps which are regarded as inventive and therefore, they also enjoy inventive step. Therefore, the requirements of Article 56 EPC are fulfilled.

Article 83 EPC

35. There is no objection on file as to the reproducibility of the claimed subject-matter relating to the claimed methods/use carried out with double-stranded polynucleotide variants. The board is also of the opinion that there is sufficient information in the

patent specification for claimed methods/use to be reproduced without undue burden.

Order

For these reasons, it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent in amended form with the following claims and a description to be adapted:

Claims

No. 1 to 27 received during oral proceedings of 12 December 2007.

The Registrar The Chairman

A. Wolinski L. Galligani