BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:
(A) [ ] Publication in OJ
(B) [ ] To Chairmen and Members
(C) [ ] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 18 October 2007

Case Number: T 1188/06 - 3.3.08

Application Number: 98901380.0

Publication Number: 0988378

IPC: C12N 15/10

Language of the proceedings: EN

Title of invention:
A method for in vitro molecular evolution of protein function

Patentee:
BioInvent International AB

Opponents:
MAXYGEN INC.
NOVOZYMES A/S

Headword:
Molecular evolution/BIOINVENT

Relevant legal provisions:
EPC Art. 54, 56, 83, 123(3)

Keyword:
"Main request - extension of protection - yes"
"Auxiliary request 1 - novelty - yes"
"Inventive step - yes"
"Sufficiency of disclosure - yes"

Decisions cited:
T 0019/90, T 0187/91, T 0210/93

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1188/06 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 18 October 2007

Appellant: (Patent Proprietor)	BioInvent International AB Sölvegatan 41 SE-223 70 Lund (SE)
Representative:	Thomas, Philip John Duval Eric Potter Clarkson LLP Park View House 58 The Ropewalk Nottingham NG1 5DD (GB)
Respondent: (Opponent 1)	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)
(Opponent 2)	NOVOZYMES A/S Krogshoejvej 36 DK-2880 Bagsvaerd (DK)
Representative:	Power, David J.A. Kemp & Co. 14 South Square Gray's Inn London WC1R 5JJ (GB)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted 26 May 2006 revoking European patent No. 0988378 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
C. Rennie-Smith

Summary of Facts and Submissions

I. The patentee (appellant) lodged an appeal against the decision of the opposition division dated 26 May 2006, whereby European patent 0 988 378 was revoked. The patent had been granted on European patent application No. 98 901 380.0 entitled "*A method for in vitro molecular evolution of protein function*" and published under the international publication number WO 98/32845.

II. The patent had been opposed by two opponents. The grounds for opposition relied on were lack of novelty (Article 100(a) EPC), lack of inventive step (Article 100(a) EPC) and insufficiency of disclosure (Article 100(b) EPC).

III. Basis for the revocation were the claims as granted (main request) and eleven auxiliary requests. The main request was refused for reason of lack of novelty of claim 3 over document D12 (see Section IX, infra). Auxiliary requests 1 and 11 were considered not to involve an inventive step in view of document D3 (see Section IX, infra) taken as the closest prior art in combination with any one of the numerous documents relating to gene splicing by overlap extension methodology. Auxiliary requests 2 to 10 were found to lack novelty vis-à-vis document D13 (see Section IX, infra).

IV. Together with its statement setting out the grounds of appeal dated 25 September 2006, the appellant filed a new main request and 14 auxiliary requests (1 to 14) to replace all the claim requests on file.

V. Each of the two respondents (the two opponents) filed observations on the statement setting out the grounds of appeal in letters dated 21 February 2007.

VI. The Board issued a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal in which provisional and non-binding opinions were expressed.

VII. With a letter dated 17 September 2007, the appellant filed additional submissions as well as a new main request and ten new auxiliary requests (1 to 10) to replace all previous requests.

Claim 1 of the main request was derived from independent claim 17 as granted and read as follows with omissions shown in square brackets and additions in bold:

"1. A method of creating a polynucleotide library comprising the steps of obtaining [a] parent polynucleotide**s each** encoding one or more variant protein motifs;

a) providing a plurality of pairs of oligonucleotides, each pair representing spaced apart locations on the parent polynucleotide sequence**s** bounding an intervening variant protein motif, and using each said pair of oligonucleotides as amplification primers for PCR to amplify the intervening motif;
b) obtaining single-stranded nucleotide sequences from the thus-isolated amplified nucleotide sequences;

c) assembling polynucleotide sequences by incorporating nucleotide sequences derived from step b) above with nucleotide sequences encoding scaffold sequences; and
d) inserting said polynucleotide sequences into suitable vectors."

Auxiliary request 1 consisted of 8 claims corresponding identically (except for the renumbering and adaptation of back-references) to claims 17 to 20 and claims 23 to 26 as granted. Claim 1 read as follows:

"1. A method of creating a polynucleotide library comprising the steps of obtaining a parent polynucleotide encoding one or more variant protein motifs;

a) providing a plurality of pairs of oligonucleotides, each pair representing spaced apart locations on the parent polynucleotide sequence bounding an intervening variant protein motif, and using each said pair of oligonucleotides as amplification primers for PCR to amplify the intervening motif;
b) obtaining single-stranded nucleotide sequences from the thus-isolated amplified nucleotide sequences;
c) assembling polynucleotide sequences by incorporating nucleotide sequences derived from step b) above with nucleotide sequences encoding scaffold sequences; and
d) inserting said polynucleotide sequences into suitable vectors."

Claims 2 to 8 of both requests were dependent on claim 1 and directed to particular embodiments thereof.

VIII. Oral proceedings took place on 18 October 2007.

IX. The following documents are referred to in the present decision:

(D3) WO 97/08320 published on 6 March 1997

(D9) Eskil Söderlind et al., Gene, Vol. 160, 1995, Pages 269 to 272

(D12) Robert M. Horton et al., Gene, Vol. 77, 1989, Pages 61 to 68

(D13) WO 98/27230 published on 25 June 1998 with a priority date of 18 December 1996

(D14) WO 98/42832 published on 1 October 1998 with a first priority date of 25 March 1997

(D25) Robert M. Horton et al., Methods in Enzymology, Vol. 217, 1993, Pages 270 to 279

(D29) P. Jirholt et al., Gene, Vol. 215, 1998, Pages 471 to 476

X. The submissions made by the appellant (patentee), insofar as they are relevant to the present decision, may be summarised as follows:

Main request (Article 123(3) EPC)

The use of more than one parent polynucleotide as a source of variant protein motifs was an inherent feature of claim 17 as granted. Therefore, the

replacement in claim 17 as granted of the expression "a parent polynucleotide" by the expression "parent polynucleotides" had not extended the protection conferred by the patent. The reasoning in decision T 187/91 of 11 March 1993 applied to the present case.

Auxiliary request 1

Novelty (Article 54 EPC)

Document D12 reported the use of gene splicing by overlap extension to construct a recombinant gene encoding a mosaic fusion protein comprised of parts of two different mouse class-I major histocompatibility genes. It did not disclose a method of creating a polynucleotide library.

The method of document D13 differed from the method of claim 1 at least in that there was no amplification of the parent polynucleotides and bridge primers were used. The disclosure in the only specific passage in the document (see Section (G) on page 71) referring to the "gentle" fine grain search was not clear. As a result, there was no direct and unambiguous disclosure of the method of claim 1.

As for document D14, the mention of "two genes" in the legend to Figure 2 therein could not be seen as a direct and unambiguous disclosure of a polynucleotide encoding a variant protein motif. Furthermore, Figure 2 did not illustrate the production of a polynucleotide library, but of only two variant sequences. In the method of Example 3 there was no amplification of an intervening variant protein motif. This was evident

from page 21, lines 10 to 18 which described the product as "a large smear", i.e. not one amplified sequence. As derivable from Figure 5, there was no amplification of the two parent polynucleotides.

Inventive step (Article 56 EPC)

Document D9 focused on the construction entirely *in vitro* of totally synthetic gene libraries for the variable light chain. The intention of the authors was to find a method which did not depend on *in vivo* pre-formed antibody specificities. The whole concept of the method of document D9 was to make a synthetic library by use of randomised oligonucleotides. It was an alternative to methods which involved pre-existing variant sequences. Thus, document D9 taught away from the use of regions from pre-formed sequences. The skilled person looking for a source of variant sequences would not have replaced the chemical synthesis of the method of document D9 by an amplification of pre-formed variant sequences obtained from a parent polynucleotide.

Sufficiency of disclosure (Article 83 EPC)

It was not derivable from document D29 that the use of a member of a specific binding pair, such as biotin, was critical to obtain single-stranded sequences. In this respect, document D29 failed to provide any serious doubts, substantiated by verifiable facts. The respondents failed to explain why obtaining single-stranded sequences would cause a skilled person any difficulty whatsoever.

XI. The submissions made by respondent I (opponent 1), insofar as they are relevant to the present decision, may be summarised as follows:

Main request (Article 123(3) EPC)

No objection was made.

Auxiliary request 1

Novelty (Article 54 EPC)

Claim 1 was not new over document D12 which disclosed the basic SOEing methodology (gene Splicing by Overlap Extension), an approach for recombining DNA molecules at precise junctions without the use of restriction endonucleases or ligase. All the steps of the method of claim 1 were indicated in Figure 1 (see page 63). This was in particular the case for the step of "obtaining single-stranded nucleotide sequences", account being taken of the fact that single-stranded nucleotide sequences were always produced as a first step in oligo-directed recombination reactions (as shown in Figure 2 on page 64 of document D12).

Claim 1 was not new over the disclosure in document D13 of a "gentle fine grain" method for evolution of proteins and its application to interferon alpha. The object of the method was the creation of a polynucleotide library (see page 71, lines 15 to 17). Each of the nine degenerate oligonucleotides was amplified by PCR. Thus, it involved a step of providing a plurality of pairs of oligonucleotides, as in claim 1, and a step of obtaining single-stranded nucleotide

sequences, because single-stranded sequences were inevitably produced during the cycling steps of denaturation, reannealing and primer extension used in PCR. Full-length genes were generated using the oligonucleotide directed recombination method (see page 70). This was the same assembly method as used in claim 1. Thus, the "gentle fine grain" method included the step of "assembling polynucleotide sequences" of claim 1. It also provided the claimed step of "obtaining single-stranded nucleotide sequences", because single-stranded nucleotide sequences were always produced as a first step in oligo-directed recombination reactions.

As for document D14, Example 3 therein described the production and screening of multiple recombinant sequences. The method was schematically illustrated in Figure 2. Two pNB esterase genes were recombined using the "defined primer" recombination technique. This was a disclosure of a method of creating a polynucleotide library as required by claim 1.

Inventive step (Article 56 EPC)

Either of documents D3 and D9 could be chosen as the closest prior art. The method of creating a polynucleotide library described in document D9 differed from the method of claim 1 only in the source of the variant sequences. In the method of document D9, the variant sequences were chemically synthesized whereas in the method of claim 1 they were amplified pre-formed sequences taken from a parent polynucleotide. From common general knowledge the skilled person would have regarded it as obvious to replace in the method of

document D9 the chemical synthesis of the variant sequences by such an amplification synthesis.

Sufficiency of disclosure (Article 83 EPC)

The presence of a member of a specific binding pair (MSBP) such as biotin linked to one member of each primer pair was the only way indicated by the opposed patent for isolating the single-stranded nucleotide sequences referred to in step b) of claim 1. As the patent did not indicate how methods not involving any MSBP could be carried out, it failed to provide an enabling disclosure.

XII. The submissions made by respondent II (opponent 2), insofar as they are relevant to the present decision, were essentially the same as those made by respondent I. Additional comments were made which can be summarized as follows:

Main request (Article 123(3) EPC)

The replacement in claim 1 of the expression "a parent polynucleotide" by the expression "parent polynucleotides" resulted in the extension of the protection conferred by the patent. The source of variant protein motifs and of scaffolds had been diversified.

Auxiliary request 1

Novelty (Article 54 EPC)

As regards document D12, the reported use of gene splicing by overlap extension to construct a recombinant gene was relevant even if the creation of a polynucleotide library was not referred to therein because in the characterising part of claim 1 there was no step indicating how the library referred to in its preamble was to be prepared. Furthermore, the reasoning of decision T 210/93 of 12 July 1994 applied when assessing novelty over document D12.

As regards document D13, its disclosure was not limited to interferon alpha on which Example 3 was focused but was concerned with polypeptides in general. Thus, it provided a general disclosure of the method of claim 1.

For the same reasons indicated by respondent I, claim 1 lacked novelty also over document D14.

Inventive step (Article 56 EPC)

Starting from either document D3 or document D9, chosen as the closest prior art, the person skilled in the art would have regarded it as obvious to modify the method described therein by using the gene splicing by overlap extension technology as described in particular in document D12 or document D25.

Sufficiency of disclosure (Article 83 EPC)

For the same reasons indicated by respondent I, the method of claim 1 was not sufficiently disclosed.

XIII. The appellant (patentee) requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request or one of the auxiliary requests 1 to 10 filed on 17 September 2007.

XIV. The respondents (opponents) requested that the appeal be dismissed.

Reasons for the Decision

1. *Main request*

1.1 Claim 1 of the main request, which is directed to a method of creating a polynucleotide library, differs from claim 17 as granted from which it derives (see Section VII, supra) in that its starting point is no longer a step of obtaining "a parent polynucleotide" but a step of obtaining "parent polynucleotides". Because each parent polynucleotide is *per se* a source of variant motifs and possibly of scaffolds, the method of claim 1 allows the creation of a library containing a multiplicity of polynucleotides which cannot be obtained with the method of claim 17 as granted. Thus, this is a marked difference which has resulted in an extension of the scope of the protection conferred by the patent, a further consideration being that none of the other independent claims as granted can be read in

the plural since they use the wording "a parent polynucleotide".

1.2 In support of claim 1, the appellant argues that the rationale of decision T 187/91 (see Section X, supra) applies in the present case. However, in that decision the Board had to deal with an issue of added matter under Article 123(2) EPC, not an issue of extension of protection under Article 123(3) EPC. Therefore, the argument is not tenable.

1.3 Thus, claim 1 contravenes Article 123(3) EPC and the main request is refused.

2. *Auxiliary request 1*

2.1 Novelty (Article 54 EPC)

2.1.1 Claim 1 is directed to a method for creating a polynucleotide library which comprises a number of steps, namely the step of obtaining a parent polynucleotide and steps (a) to (d) which are essential to its performance.

2.1.2 Three documents, namely D12, D13 and D14, are cited against claim 1. Documents D13 and D14 are cited under Article 54(3) EPC in view of the fact that claim 1 is admittedly only entitled to 26 January 1998 as its filing date and not to 24 January 1997, the claimed priority date, as the priority document covers only a method in which one of the pairs of oligonucleotides provided in step a) is always linked to a member of a specific binding pair (MSBP), a feature which is absent from claim 1.

2.1.3 Document D12 describes the use of a polymerase chain reaction-based approach to genetic engineering called "gene Splicing by Overlap Extension" (SOE) which does not depend on the occurrence of restriction enzyme recognition sequences at the recombination site to construct a gene encoding a mosaic fusion protein comprised of parts of two different mouse class-I major histocompatibility genes. The ambit of document D12 does not go beyond the construction of individual genes. The general mechanism of SOE as illustrated in Figure 1 (see page 63) involves a succession of steps which allows the construction of a particular recombinant product and its amplification as soon as it is formed. There is no disclosure of a method for creating a polynucleotide library.

2.1.4 Respondent II argues that nevertheless the rationale of decision T 210/93 (see Section XII, supra) should apply to the present case. This argument is not tenable as there is no disclosure in document D12 of a method inevitably leading to a polynucleotide library. Although the SOE technology was known at the filing date, there is no information in document D12 from which it could be derived that a polynucleotide library would be inevitably obtained, even when considering the mere speculative statement found at the top of page 62 that "[T]he SOE approach is a fast, simple, and extremely powerful way of recombining and modifying nucleotide sequences".

2.1.5 Thus, the method of claim 1 is new over document D12.

2.1.6 Document D13 describes methods for polypeptide engineering relying on recursive sequence recombination and involving one of two search strategies classified as "coarse grain shuffling" and "fine grain shuffling" which allow analysis of variation occurring within a nucleic sequence. Section (E) (see page 70) describes how to carry out a fine grain search when looking for improved interferon (IFN) alpha hybrids. The modelled structure of IFN alpha has been divided into nine adjacent and non-overlapping segments (see Table III on page 69). Each of the nine segments is synthesized as well as two sets of degenerate oligonucleotides encoding the nine segments. Each of the nine synthetic segments is then amplified by PCR with the 18 PCR oligonucleotides. Full length genes using the oligo direct recombination method are generated, transfected into a host, and assayed for hybrids with desired properties.

2.1.7 The respondents argue that Section (G)) (see page 71) describes a particular embodiment of the fine grain shuffling which corresponds to the method according to claim 1.

2.1.8 In reality, Section (G) is only a brief (8 lines) and merely speculative (as the use of the conditional tense underlines) passage of the description which only suggests to make the fine grain search as reported in section (E) more "gentle", this being supposedly achieved by obtaining a candidate starting point, such as the "IFN-Con1 consensus interferon" and "gently" searching from there. This disclosure is insufficient to provide the skilled person with a clear view of how Section (G) actually combines with Section (E). Thus,

the information provided by Section (G) does not amount to a clear and unambiguous disclosure as required for an assessment of novelty. Therefore, claim 1 is new over document D13.

2.1.9 Document D14 describes a method for *in vitro* mutagenesis and recombination of polynucleotide sequences using interspersed internal "defined primers". (see Figure 2 in which the method is schematised). Rather than reassembling recombined genes from a fragment pool, the "defined primer" method prepares full-length recombined genes in the presence of templates by a process, designated as the "staggered extension" process (StEP). This process consists of priming the template sequences followed by repeated cycles of denaturation and extremely abbreviated annealed/polymerase-catalysed extension. In each cycle the growing fragments anneal to different templates based on sequence complementarity and extend further. This is repeated until full-length sequences form. The method is illustrated in Example 3 with describes the recombination of two pNB esterase genes (see also Figure 5).

2.1.10 A comparison of the "defined primer" method of D14 with the method of claim 1 immediately reveals a major difference therebetween. Whereas the method of claim 1 at issue starts from a parent polynucleotide, the "defined primer" method of document D14 requires as its starting material the presence of (at least) two different parent polynucleotides, as evidenced by Figure 2 (see the comments thereon made from line 32 on page 5 to line 3 on page 6) and Example 3, in which two template pNB esterase genes, referred to as 2-13 and

5-B12, were recombined (see also Figure 5 together with the comments thereon made on page 6, lines 24 to 31). This difference is in itself sufficient to establish novelty of the method of claim 1 over document D14.

2.1.11 Thus, claim 1 meets the requirements of Article 54 EPC. The same conclusion applies *de facto* to the particular embodiments covered by dependent claims 2 to 8.

2.2 Inventive step (Article 56 EPC)

2.2.1 Each of documents D3 and D9 has been taken as the closest prior art in the decision under appeal.

2.2.2 According to established jurisprudence of the EPO Boards of appeal the closest prior art for assessing inventive step is normally 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.

2.2.3 Document D3 discloses a method that enables the creation of useful libraries of polypeptides (see page 5, line 2), which can be expressed in a suitable vector and screened for a desired property (see page 7, lines 5 to 22). The method is primarily based on a bioinformatics approach. It includes the computer-aided design of a limited set of synthetic nucleic acid consensus sequences. Starting from the identification and analysis of a collection of at least three homologous proteins, a database is established in which the individual sequences are aligned to each other. For each subgroup of protein sequences, a polypeptide

consensus sequence is deduced. These artificial polypeptide consensus sequences are analysed to identify unfavourable interactions which are then removed by altering the consensus sequences accordingly. Then the artificial polypeptide consensus sequences are each back-translated into a corresponding nucleic acid sequence and a set of cleavage sites is set up in each nucleic acid subsequence encoding a structural element (see page 5, first paragraph and claim 1). These cleavage sites are essential to generate new nucleotide sequences. They are used to excise and replace modules with a different sequence compatible with the cleaved nucleic acid.

2.2.4 Document D9 discloses a method for producing a completely synthetic gene library encoding the variable light (VL) immunoglobulin domains (see the abstract on page 269). The method allows cloning and selection of the antibody fragments in any phage display system (see page 270, left-hand column).

2.2.5 Whereas, as explained at point 2.2.9 (see infra), the method of document D9 differs from the method of claim 1 only in that the step of obtaining the variant protein motifs relies on a different technology, in stark contrast, as outlined at point 2.2.3 (see supra), the method of document D3 is based on a fundamentally different concept, according to which not a parental polynucleotide serves as the starting point but a series of computer-aided designed nucleic acid sequences incorporating cleavage sites.

2.2.6 Thus, although the methods disclosed in both documents D3 and D9 belong to the same technical field as the

claimed method (creation of a polynucleotide library) and are directed to a similar purpose or effect as the invention (increasing genetic variation with the objective of selecting proteins with desired characteristics), not document D3 but document D9 represents the closest state of the art.

2.2.7 The method of document D9 is illustrated schematically in Figure 2. A 374-bp sequence, covering the entire variable light (VL) domain of a known anti-lysozyme antibody, was divided into six regions represented by six synthetic oligodeoxyribonucleotides (see the six overlapping internal primers L1 to L6 of Figure 2). Internal primers L2, L3 and L5 were synthesized with randomized complementary determining regions (CDRs). The framework regions were unaltered. They correspond to internal primers L1, L4 and L6. Furthermore, two flanking primers (see the two amplification primers of Figure 2) were used in the assembly process to permit desired restriction sites to be engineered into the synthetic libraries and allow cloning and selection of antibody fragments in any phage display system. The synthetic library for the VL domain could be assembled in one single PCR step.

2.2.8 Thus, document D9 teaches a method of creating a polynucleotide library in which the variant motifs, represented by the internal primers L2, L3 and L5, are produced by chemical synthesis. The variant protein motifs are assembled with the scaffold sequence, represented by internal primers L1, L4 and L6 by PCR. Accordingly, steps (b), (c) and (d) of the method of claim 1 are disclosed in combination in document D9.

2.2.9 The method of claim 1 differs from the method of document D9 in that the variant sequences are obtained by amplification of nucleotide sequences from a parent polynucleotide encoding variant protein motifs, rather than by chemical synthesis.

2.2.10 The objective technical problem for the skilled person starting from the method of document D9 is the provision of an alternative method for creating a polynucleotide library. The solution to that problem is a method in which the variant sequences are obtained by amplification from a parent polynucleotide encoding variant protein motifs.

2.2.11 The question to be solved for the assessment of inventive step is whether, in order to provide variant sequences, the skilled person would have been prompted at the relevant filing date to replace in the method of document D9 the chemical synthesis by an amplification of pre-formed variant motifs taken from a parent polynucleotide.

2.2.12 Respondent I argues that the skilled person equipped with common general knowledge would have substituted amplification synthesis for the chemical synthesis. The Board is not convinced. Indeed, on the contrary, the skilled person scrutinising the statement on page 269 (right hand column) of document D9 which reads: "*Instead of depending on in vivo preformed Ab specificities found in gene libraries, we have investigated an alternative route for the design and construction of V-region libraries*" would have realised that the whole concept of the document was to **construct entirely *in vitro* synthetic gene libraries** for the

variable light chain. Thus, document D9 teaches away from use of pre-formed sequences.

2.2.13 Respondent II argues that either document D12 (see point 2.1.3, supra) or document D25 (which provides a basic detailed description of the gene splicing by overlap extension) would have prompted the skilled person to replace the chemical synthesis in the method of document D9 by an amplification of pre-formed variant motifs taken from a parent polynucleotide. The Board is not convinced. As explained above (see point 2.2.12, supra) the skilled person interested in the alternative route for the design and construction of V-region gene libraries described in document D9 would have had no reason to pay attention to any document which as document D12 or document D25 described a polymerase chain reaction-based technology.

2.2.14 For these reasons the skilled person would have found no suggestion to substitute an amplification synthesis for the chemical synthesis of document D9.

2.2.15 Therefore, the method according to claim 1 involves an inventive step. The same conclusion applies *de facto* to the particular embodiments covered by dependent claims 2 to 8.

2.3 Sufficiency of disclosure (Article 83 EPC)

2.3.1 The method of claim 1 inherently comprises a step of isolating the amplified nucleotide sequences (see the reference in step b) to "the thus-isolated amplified nucleotide sequences").

2.3.2 Actually, the description, in both its general and experimental parts, indicates that those sequences may be isolated using members of a specific binding pair (MSBPs), such as biotin and avidin, (see page 6, lines 4 to 10; page 9, lines 6 to 10; page 10 (comments on Figure 5); page 11 (comments on Figure 8); page 13, lines 30 to 32; page 18, lines 25 to 28; and page 20, lines 2 to 6).

2.3.3 Whereas it can be concluded that the description describes in detail at least one way of carrying out the isolation of the amplified nucleotide sequences referred to in step b) of claim 1, the respondents argue that later document D29 (expert opinion) shows that the method of claim 1 cannot be put into effect without linking one of each pair of oligonucleotides as referred to in step a) of the claimed method to a MSBP, such as biotin.

2.3.4 Document D29 is a post-published document to which the inventors of the patent contributed. It illustrates the construction of a particular gene library encoding soluble domains of the variable region of an immunoglobulin heavy chain using a master framework together with *in vivo* formed PCR amplified complementary determining regions.

2.3.5 Whereas in the particular process described in document D29, use of biotin is made to obtain single stranded nucleotide sequences, it is not stated that such a use is essential and that those sequences could not be isolated using another MSBP or another technology. Thus, document D29 does not provide any serious doubts, substantiated by verifiable facts - see decision

T 19/90 (OJ EPO 1990, 476) - and the respondents' argument fails.

2.3.6 Therefore, the method according to claim 1 is sufficiently disclosed. As the sufficiency of disclosure of the embodiments of the invention according to dependent claims 2 to 8 has not been questioned, it is concluded that auxiliary request 1 as a whole meets the requirement of Article 83 EPC.

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 on the basis of the auxiliary request 1 filed on 17 September 2007 and a description and drawings to be adapted thereto

The Registrar The Chairman

A. Wolinski L. Galligani