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Datasheet for the decision of 20 March 2014

Case Number: T 1053/09 - 3.3.08

Application Number: 99948721.8

Publication Number: 1124949

IPC: C12N15/11

Language of the proceedings: EN

Title of invention:
Constructing and screening a DNA library of interest in filamentous fungal cells

Patent Proprietor:
Novozymes A/S

Opponent:
Danisco US Inc.

Headword:
DNA library in filamentous fungal cells/NOVOZYMES

Relevant legal provisions:
EPC Art. 54, 56, 83
RPBA Art. 13(1)

Keyword:
"Admission into the proceedings of new evidence (yes)
Admission of main request (yes)
Novelty (yes)
Sufficiency of disclosure (yes)
Inventive step (yes)"

Decisions cited:

Catchword:

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Case Number: T 1053/09 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 20 March 2014

Appellant: (Opponent)	Danisco US Inc. 925 Page Mill Road Palo Alto CA 94304-1013 (US)
Representative:	Kremer, Simon Mark Mewburn Ellis LLP 33 Gutter Lane London EC2V 8AS (GB)
Respondent: (Patent Proprietor)	Novozymes A/S Krogshøjvej 36 2880 Bagsvaerd (DK)
Representative:	Didmon, Mark Potter Clarkson LLP The Belgrave Centre Talbot Street Nottingham, NG1 5GG (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 4 March 2009 concerning maintenance of the European Patent No. 1124949 in amended form.**

Composition of the Board:

Chairman	M. Wieser
Members:	M. R. Vega Laso
	C. Heath

Summary of Facts and Submissions

I. European patent No. 1 124 949 with the title "Constructing and screening a DNA library of interest in filamentous fungal cells" was granted on European patent application No. 99948721.8, which was filed as international application PCT/DK99/00552 and published as WO00/24883 (in the following "the application as filed"). The patent was granted with 29 claims.

II. An opposition to the grant of the patent was filed based on the grounds for opposition under Article 100(a) and (b) EPC, in particular that the claimed subject-matter lacked novelty (Article 54 EPC) and an inventive step (Article 56 EPC), and that the claimed invention was not disclosed in the patent in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

III. By an interlocutory decision under Article 101(3)(a) and 106(2) EPC posted on 4 March 2009, the opposition division found that Article 100(a) EPC prejudiced the maintenance of the patent as granted because the subject-matter of claims 24 to 29 lacked novelty over documents (1), (2) and (5) (see section XIII below). However, the opposition division held that, in view of the amendments introduced into the set of amended claims according to the auxiliary request 2 filed by letter dated 17 September 2008, and into pages 3, 12 and 13 of the description as filed during the oral proceedings, the patent in amended form and the invention to which it related met the requirements of the EPC.

IV. The opponent (appellant) lodged an appeal against the decision of the opposition division and filed a statement of grounds of appeal together with new evidence (documents (16) and (17); see section XIII below).

V. By letter dated 17 November 2009, the patent proprietor (respondent) filed a reply to the statement of grounds of appeal including new evidence (document (18); see section XIII below) and five sets of claims as auxiliary requests 1 to 5. The set of claims according to the auxiliary request 2 underlying the decision under appeal was maintained as main request.

VI. In a further submission, the appellant provided observations on specific arguments of the respondent.

VII. As a subsidiary request, both parties requested oral proceedings.

VIII. The parties were summoned to oral proceedings. In a communication under Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) attached to the summons, the board made observations on the admissibility of requests and evidence submitted in appeal proceedings, and expressed a provisional opinion on some of the substantive issues to be discussed during the oral proceedings, in particular issues in connection with Articles 123(2), 83, 54 and 56, and Rule 80 EPC.

IX. By letter dated 19 February 2014 the respondent replied to the board's communication. It submitted four sets of claims as, respectively, new auxiliary requests 2 to 5, and renumbered the sets of claims of the auxiliary

requests 2 to 5 filed on 17 November 2009 as auxiliary requests 6 to 9.

X. The appellant submitted observations on the board's communication.

XI. Oral proceedings were held on 20 March 2014. After the discussion of the main request and auxiliary requests 1 and 2 then on file, the respondent re-filed the set of claims according to auxiliary request 2 as its new main request.

XII. Claims 1, 21 and 23 of the **main request** read:

"1. A method of constructing and selecting or screening a library of polynucleotide sequences of interest in filamentous fungal cells wherein the method comprises:

a) transforming the fungal cells with a population of DNA vectors, wherein each vector comprises:

i) a polynucleotide sequence encoding a fungal selection marker and a fungal replication initiating sequence wherein the marker and the replication initiating sequence do not vary within the population; and

ii) a polynucleotide sequence of interest wherein the population of DNA vectors contains more than one variant of the polynucleotide sequence;

b) cultivating the cells under selection pressure;

c) selecting or screening for one or more transformants expressing a desired characteristic; and

d) isolating the transformant(s) of interest,

and wherein the replication initiating sequence is a nucleic acid sequence having at least 80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2 and is capable of initiating replication.

21. A method of constructing and screening or selecting a library of polynucleotide sequences of interest in a filamentous fungal cell, wherein the method comprises:

(a) transforming a culture of bacterial or yeast cells with a population of the DNA vectors as described in any of claims 1-20, wherein the vector further comprises a nucleic acid sequence encoding a bacterial or yeast selective marker and a bacterial or yeast replication initiating sequence;
(b) cultivating the bacterial or yeast cells under selection pressure;
(c) isolating the DNA constructs from the transformants of (b);
(d) transforming filamentous fungal cells with the DNA constructs of (c);
(e) cultivating the filamentous fungal cells of (d);
(f) selecting or screening for one or more filamentous fungal transformants expressing a desired characteristic; and
(g) isolating the filamentous fungal transformant(s) of interest.

23. Use of a fungal replication initiating sequence in the construction of a library of polynucleotide sequences of interest in a method according to any of claims 1 to 22, and wherein the fungal replication initiating sequence is a nucleic acid sequence having at least 80% identity with the nucleic acid sequence of

SEQ ID NO:1 or SEQ ID NO:2 and is capable of initiating replication."

Dependent claims 2 to 20 and 22 are directed to particular embodiments of the method of claim 1 and claim 21, respectively. Dependent claims 24 and 25 specify additional features of the use according to claim 23.

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

(1): D. H. Gems and A. J. Clutterbuck, 1993, Curr. Genet., Vol. 24, pages 520 to 524;

(2): A. Aleksenko and A. J. Clutterbuck, 1997, Fungal Genetics and Biology, Vol. 21, pages 373 to 387;

(3): A. Aleksenko et al., 1996, Molecular Microbiology, Vol. 20, No. 2, pages 427 to 434;

(4): A. Aleksenko et al., 1996, Mol. Gen. Genet., Vol. 253, pages 242 to 246;

(5): J. C. Verdoes et al., 1994, Gene, Vol. 146, pages 159 to 165;

(6): J.-H. Zhang et al., April 1997, Proc. Natl. Acad. Sci. USA, Vol. 94, pages 4504 to 4509;

(7): L. You and F. H. Arnold, 1994, Protein Engineering, Vol. 9, No. 1, pages 77 to 83;

(9): T. C. Huffaker, 1993, Methods in Enzymology, Vol. 217, pages 301 to 312;

(15):Experimental report "Creation and screening of a library of polynucleotide sequences in filamentous fungal cells involving the transformation of pre-formed vectors"

(16):J. Rambosek and J. Leach, 1987, in ????, Vol. 6, Issue 4, pages 357 to 393;

(17):D. J. Balance, 1986, Yeast, Vol. 2, pages 229 to 236;

(18):Experimental report "Further testing of variants identified by screening of polynucleotide sequences in filamentous fungal cells".

XIV. The submissions made by the appellant concerning issues relevant to this decision, were essentially as follows:

Admission of new evidence into the proceedings

Document (18) had been filed at a late stage of the proceedings and should not be admitted.

Main request - Admission into the proceedings

With the new set of amended claims the respondent addressed the objection of lack of sufficient disclosure in the patent which had been raised already in the notice of opposition. Since there was no reason why the amended claims could not have submitted earlier, they should not be admitted into the proceedings.

Article 83 EPC – Sufficiency of disclosure

A method using AMA1 (SEQ ID NO:1) as replication initiating sequence was the sole credibly disclosed method in the application as filed for providing uniform transformants and a stable and standard environment for gene expression, as required for *effective* comparison of variants of a single polypeptide.

As regarded the MATE element (SEQ ID NO:2), the claimed invention could not be carried out without an undue burden of experimentation. Although AMA1 consisted of two copies of MATE1 and a functionally inert spacer, the properties of MATEs were very different from those of AMA1. Plasmids bearing single MATE elements acted in transformation much less effectively than the AMA1 duplication (see document (2), page 379, left-hand column, lines 2 and 3 from the bottom). Moreover, it was stated in document (3) (see "Summary") that, even though a single copy of a MATE element increased transformation frequency to a modest extent, it led to multiple rearrangement, unstable integration or concatenation of vector molecules.

Article 54 EPC – Novelty

The opposition division erred in finding the subject-matter of claims 1 to 23 novel. They had arrived at this conclusion because they considered the claims to exclude vectors formed by *in vivo* recombination, and also because they considered that the claims required that different vectors contain different sequences. However, such an interpretation of the claims was not based on its wording, nor even on a fair reading of the description.

Document (1) contained two distinct teachings, one of them referring to co-transformation of two plasmids that recombined *in vivo*. The method as claimed in claim 1 required that cells were transformed with a population of DNA vectors, but, since the claim did not specify how those DNA vectors were provided, *in vivo* recombination as described in document (1) was encompassed. This was confirmed by the description of the patent, in particular by Example 4 which was expressly said to be an embodiment of the invention. Thus, the subject-matter of claim 1 lacked novelty over document (1).

Furthermore, document (1) described direct transformation of fungal cells with a vector (pDHG8) which contained a fungal selection marker (argB gene), AMA1 as fungal replication initiating sequence, and polynucleotide sequences of interest (adC and adD genes). Claim 1 did not require that each vector contained a variant of the polynucleotide sequence; step (a) of the claim required transformation with a "vector population", not with individual vectors and not with a "library". Document (1) was also concerned with "libraries". Thus, also with regard to this aspect, claim 1 lacked novelty.

Claim 21 required that the vector was also apt for screening in a bacterial host, as recited in additional steps (a) to (d). It was described on page 523, lines 1 to 8 of document (1) that plasmid pDHG8 was introduced into *E. coli* and replicated in this organism under selective pressure. This implied that pDHG8 had an origin of replication and a selection marker. Plasmid DNA prepared from one of the *E. coli* transformants was used for transformation of *A. nidulans*. Thus, the

additional steps in claim 21 were all disclosed in document (1) and could not render the claim novel over this document.

Article 56 EPC – Inventive step

The claimed subject-matter did not involve an inventive step within the meaning of Article 56 EPC.
Document (6), which was regarded as the closest state of the art, taught every aspect of the claimed invention except that bacterial host cells and appropriate marker and replication sequences were used for expressing the genes in the library.

Starting from document (6), the objective technical problem was to modify the bacterial system described therein to provide an alternative method of generating a variant polynucleotide sequence of interest which could be (definitely) expressed in a filamentous fungal production host.

It was already known long before the priority date that attempts to express active filamentous fungal genes in bacterial (and yeast) hosts often failed because they could not process fungal genes properly. By contrast, attempts to express filamentous fungal genes in other filamentous fungi were more successful (see documents (16) and (17)).

Therefore, a person skilled in the art would have been directed towards filamentous fungal hosts as being a promissing solution to the problem of generating variant polynucleotide sequences of interest which can be (definitely) expressed in a filamentous fungus.
Furthermore, document (2) and, *inter alia*, document (4) showed that fungal expression systems were available at

the priority date – indeed several such systems were adopted in the patent. Thus, the skilled person had both the motivation and the means to adopt the claimed invention at the priority date.

XV. The submissions made by the respondent, as far as they related to the decisive issues, may be summarized as follows:

Main request – Admission into the proceedings

The set of amended claims had been submitted in reply to the board's communication under Article 15(1) RPBA. During the opposition proceedings there had never been an indication that the disclosure of the claimed invention in the patent may be insufficient, and in the decision under appeal the opposition division had found that the ground of opposition under Article 100(b) EPC did not prejudice the maintenance of the patent as granted. Thus, before the board expressed its concerns in this respect, there had been no reason to file amended claims addressing the issue.

Article 83 EPC – Sufficiency of disclosure

It had never been contested that a method as claimed in which the AMA1 sequence (SEQ ID NO:1) was used as fungal replication initiating sequence could be carried out by a person skilled in the art. As concerned the MATE element (SEQ ID NO:2), the application as filed provided sufficient information by reference to the prior art. Moreover, it was shown in Example 4 that the use of this element as replication initiating sequence allowed to screen a library of variant polynucleotides in filamentous fungal cells.

Article 54 EPC – Novelty

The subject-matter of claims 1 and 22 was novel over document (1). Claim 1 did not allow that vectors having the features specified in the claim were formed *in vivo* following transformation, as described in document (1). Rather, the method of claim 1 required that each vector to be introduced into the fungal cell comprised all the sequences specified in step (a). This applied also to claim 22 which referred to the DNA vectors described in claim 1.

It was clear from page 523 of document (1) that vector pDHG8 bore a single polynucleotide sequence of interest containing two genes (adC and adD) closely linked in the *Aspergillus* genome. However, in a population of the pDHG8 vector each and every individual copy of the vector would have the same single polynucleotide sequence, and not a polynucleotide sequence of interest that varies among the vector population, as required by claim 1, step (a)(ii).

Article 56 EPC – Inventive step

The technical problem formulated by the appellant in view of document (6) contained elements of the solution – particularly, the use of filamentous fungal host cells in the claimed method.

There was nothing in document (6) that would motivate a skilled person to replace the *E.coli* host described therein by a filamentous fungus. Document (6) was completely silent as to any alternative host cell types that could potentially be used in place of *E. coli*. Moreover, even if the skilled person were to try to replace *E. coli*, this document did not provide it with

any guidance of how to adapt that method for use with filamentous fungal host cells. Since the methods using filamentous fungal host cells described in the documents (1), (2) and (5) cited by the appellant relied on co-transformation of several vectors, the skilled person would not have arrived at the subject-matter of claim 1 in an obvious manner. Thus, an inventive step was to be acknowledged.

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

XVII. The respondent (patent proprietor) requested that the patent be maintained based on the main request as filed during oral proceedings.

Reasons for the Decision

Admission of new evidence into the proceedings

1. Documents (16) and (17) were filed by the appellant together with its statement of grounds of appeal as additional evidence in support of the objection of lack of inventive step (Article 56 EPC).

2. Document (16) was submitted as evidence for the skilled person's knowledge of the potential problems that may arise when filamentous fungal genes are expressed in *E. coli*. With this document, the appellant addressed the opposition division's finding in the decision under appeal that, starting from document (6) as the closest state of the art, the skilled person had no incentive to replace a bacterial host by a filamentous fungal host.

3. The appellant relied on document (17) to question the finding in the decision under appeal that, should the skilled person have considered using a different host cell, yeast cells would have been his/her first choice because protein expression in yeast was well-established at the priority date. Purportedly, the document shows that attempts to express filamentous fungal genes in yeast hosts often failed.

4. The board accepts that both documents were filed as a reaction to the decision under appeal. Since the respondent did not object to this evidence being considered by the board, documents (16) and (17) were admitted into the proceedings.

5. Document (18) was filed by the respondent together with its reply to the statement of grounds of appeal, as additional experimental evidence confirming the results of the experiments described in document (15) already on file. The appellant opposed to the admission of this document into the proceedings, but did not put forward any arguments to support its objection. The document was admitted into the proceedings.

Main request - Admission into the proceedings

6. The set of claims according to the main request was submitted during the oral proceedings before the board, but is identical to the claims of the auxiliary request 2 filed by the respondent together with its reply to the board's communication under Article 15(1) RPBA issued with the summons to oral proceedings (see sections IX and XI above). The appellant objected to the admission of this set of amended claims into the proceedings.

7. Pursuant to Article 13(1) RPBA, any amendments to a party's case after it has filed its grounds of appeal or reply may be admitted and considered at the board's discretion. When exercising its discretion, the board must take into account, *inter alia*, the complexity of the new subject-matter submitted, the current state of the proceedings and the need for procedural economy.

8. The board acknowledged that, since the objections under Article 83 EPC addressed by the amendments introduced into the claims of the present main request had been raised already in the notice of opposition, the respondent could, in principle, have presented the amended claims in question either during opposition proceedings or together with its reply to the appellant's statement of grounds of appeal.

9. Nevertheless, the board, exercising the discretion conferred by Article 13(1) RPBA, decided to admit and consider the present main request because, even though the amended claims were filed at a late stage of the appeal proceedings, the introduced amendments overcome the objections under Article 83 EPC raised by the appellant, and do not increase the complexity of the subject-matter submitted or give rise to any issues which the board or the other party could not reasonable by expected to deal with without adjournment of the oral proceedings (see Article 13(3) RPBA).

Articles 123(2)(3) and 84 EPC

10. At the oral proceedings, the appellant declared that it had no objections to the amended claims under Articles 123(2)(3) and 84 EPC. Nor does the board have any.

Article 83 EPC – Sufficiency of disclosure

11. It has been admitted by the appellant that the method of claim 1 can be carried out using AMA1 (SEQ ID NO:1) as replication initiating sequence. However, the appellant disputed - relying on documents (2) and (3) - that a MATE sequence (SEQ ID NO:2) would be suitable for carrying out the invention.

12. Appellant's arguments and evidence are not convincing. Firstly, it should be noted that the passages of documents (2) and (3) indicated by the appellant relate to plasmids bearing **single** MATE elements, while the method of claim 1 is not restricted to such vectors. Secondly, claim 1 specifies that the nucleic acid sequence acting as replication initiation sequence "*... is capable of initiating replication*". Although MATE elements may be less efficient than AMA1 in initiating replication, it is stated in document (2) that "*There is little doubt that MATEs are able to initiate extrachromosomal plasmid replication*" (see page 379, right-hand column, first sentence of the first full paragraph), and that "... *multiple copies of these elements provided stable replication irrespective of MATE orientation*" (see page 380, right-hand column, lines 3 to 5). Since on page 2, lines 17 to 26 of the application as filed reference is made to document (2) and its content is outlined, the board is satisfied that the skilled person has been given sufficient guidance for carrying out the claimed method using a vector bearing as replication initiation sequence a sequence having at least 80% identity with the nucleic acid sequence of SEQ ID NO:2.

Article 54 EPC – Novelty

13. In the decision under appeal, the opposition division held that, having regard to the content of documents (1) and (5), the subject-matter of claims 1 to 23 of the patent as granted was novel (see section 2.1 starting on page 4 of the decision under appeal, in particular the last paragraph on page 7 and the first on page 8).

14. In appeal proceedings, the appellant maintained its objection of lack of novelty against claims 1 and 21, which differ from claims 1 and 22 of the patent as granted in that they are limited to methods using a fungal replication initiating sequence which is at least 80% identical to the sequence of SEQ ID NO:1 or SEQ ID NO:2 and is capable of initiating replication (see section XII above).

15. The board shares the opposition division's view that document (1) does not describe a method of constructing and selecting or screening a **library** of polynucleotide sequences as defined in claim 1. The passage on page 523, first paragraph of the left-hand column of document (1) describes the transformation of a *A. nidulans* strain with a vector (pDHG8) that comprises a fungal selection marker, a fungal replication initiating sequence and a 3.6-kb insert including the *adC* and *adD* genes. However, in the board's view, *adC* and *adD* cannot be considered as variants of a single polynucleotide, but rather as two different genes.

16. Moreover, even if *adC* and *adD* were considered as two variants of a single polynucleotide, a population of pDHG8 vectors prepared as described in document (1) could not be regarded as a "library" of variants of a

polynucleotide sequence because – as the opposition division stated in the decision under appeal – the term "library" implies that the population of transformed host cells contains vectors including a polynucleotide that varies among the population. In contrast, each and every pDHG8 vector prepared as described in document (1) would contain exactly the same polynucleotide sequence.

17. The same applies, *mutatis mutandis*, as regards the method of claim 21. Thus, the objection of lack of novelty fails.

Article 56 EPC – Inventive step

18. In appeal proceedings, it was not disputed by either party that, as the opposition division found in the decision under appeal, document (6) represents the closest state of the art. However, documents (7) and (9) were cited by the appellant as alternatives.

19. Document (6) describes a method of constructing and selecting or screening a library of variants of the *lacZ* gene from *Escherichia coli*, by means of DNA shuffling and reiterative screening. As host cell, *E. coli* was used. While the native *lacZ* gene encodes a ß-galactosidase which acts only weakly on ß-D-fucosyl moieties, applying the described method a modified gene encoding an "evolved" enzyme that has enhanced ß-fucosidase activity could be isolated.

20. There was agreement between the parties that the method of claim 1 differs from the method described in document (6) in that filamentous fungal cells are used as host cells, and that each of the vectors comprising a variant or variants of the sequence of interest

includes a fungal selection marker and a fungal replication initiating sequence which is a nucleic acid sequence capable of initiating replication and having at least 80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

21. While in the decision under appeal, the opposition division formulated the objective technical problem to be solved starting from document (6) as "*... the provision of an alternative or improved method of generating and selecting a variant polynucleotide sequence of interest*", the appellant contested this finding maintaining that the problem to be solved must be formulated as "*... how to modify the method of D6 to provide an alternative method of generating a variant polynucleotide sequence of interest, which can be (definitely) expressed in a filamentous fungus*".

22. As the opposition division, the board holds that the problem formulated by the appellant contains elements of the solution proposed in claim 1, namely the use of filamentous fungal cells as host cells for expression of variant polynucleotide sequences. Document (6) neither describes nor suggests expressing a library of variants of a polynucleotide sequence in a filamentous fungus. The same is true for documents (7) and (9) which describe methods of constructing a library of variants of subtilisin in *Bacillus subtilis* (document (7)) or YFG ("your favourite gene") in yeast (document (9)).

23. Thus, the board judges that the technical problem underlying the present invention must be defined as the provision of an alternative or improved method of generating and selecting or screening a library of variant polynucleotide sequence of interest.

24. The board is convinced that this problem has been solved by the method of claim 1. For essentially the same reasons given in connection with Article 83 EPC (see paragraph 13 above), the board cannot accept the appellant's argument that the problem is not solved by a method in which each vector of the population bears a replication initiating sequence which is a nucleic acid sequence capable of initiating replication and having at least 80% identity with the nucleic acid sequence of SEQ ID NO:2.

25. As regards the question whether or not a person skilled in the art, which the appellant considered to be an industrial biotechnologist, would have any motivation to look for an alternative host, the appellant argued that a skilled person, starting from document (6) would have at least considered alternative hosts, and that filamentous fungi would be an obvious choice because they can secrete and process genes from filamentous fungi better than prokaryotic hosts.

26. The board disagrees with this view. Document (6) does not report any problems with the *E. coli* expression system that may prompt the skilled person to change the host cell. If the skilled person had nevertheless considered to try an alternative host, the board is - like the opposition division – convinced that the first choice would have been yeast. At the priority date, efficient transformation and heterologous expression methodologies were well established in yeast, while filamentous fungi were considered to be difficult host cells as regards transformation frequency and stability of the transformants.

27. In appeal proceedings, the appellant relied on the passage on page 233 of document (17) in support of its argument that attempts to express filamentous fungal genes in yeast often failed. The board remarks that, in this passage reference is made to five publications reporting successful isolation of genes from a filamentous fungus by complementation in *E. coli*; moreover, three publications are cited for successful complementation in yeast, and a further reference reports on successful expression in yeast after removal of introns and provision of a yeast promoter. In contrast, there is only one reference reporting failure of *Aspergillus* genes to be expressed in yeast (see page 233, right-hand column, first paragraph under the heading "Heterologous gene expression").

28. Hence, even the evidence provided by the appellant itself suggests that for a person skilled in the art at the priority date there was more than a reasonable expectation that a gene, in particular a filamentous fungal gene would be expressed either in *E. coli* or yeast. In contrast, very few transformation systems had been described in filamentous fungi, these systems being characterized by low transformation frequencies and difficulties in re-isolating the desired gene due to integration into the fungal genome. Even though there were some reports on autonomously-replicating plasmids in *Aspergillus* which achieved higher transformation frequencies (see documents (1), (2) and (5)), the skilled person, who has been considered by the boards of appeal to have a cautious and conservative attitude, would have tried first to construct and screen a library of polynucleotide sequences in yeast, rather than venture into the new field of filamentous fungi as host cells for expression of heterologous genes.

29. If the board were to accept that the skilled person, in spite of the above, would nevertheless have combined the teachings of document (6) with those of document (1) or document (5) in order to construct a library of variants of a polynucleotide sequence in filamentous fungal cells, the question arises why he/she would have departed from the co-transformation method using two different plasmid populations, one comprising a fungal selection marker and a fungal replication initiating sequence, and the other harbouring a library of polynucleotide sequences of interest, as described in the documents (1) and (5). The appellant has not provided a satisfactory answer to this question.

30. For these reasons, the board judges that the method of claim 1 was not obvious to a person skilled in the art. The same applies to the methods of claims 2 to 22 and the use according to claims 23 to 25.

Conclusion

31. Since the claims according to the main request and the invention to which they relate fulfil the requirements of the EPC, the patent can be maintained in amended form.

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 based on the main request filed during oral proceedings, and a description to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated