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D E C I S I O N
of 9 February 2005

Case Number: T 0340/00 - 3.3.8

Application Number: 89902422.8

Publication Number: 0395736

IPC: C12N 15/ 54

Language of the proceedings: EN

Title of invention:
Purified thermostable enzyme

Patentee:
F. Hoffmann-La Roche AG

Opponent:
Bioline (UK) Ltd.

Headword:
Thermostable enzyme/HOFFMANN-La ROCHE

Relevant legal provisions:
EPC Art. 123(2), (3), 84, 54(3), (4), 56

Keyword:
"Admissibility of a new ground of opposition on appeal (no)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
G 0010/91, T 0737/96, T 0190/99, T 1080/01

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0340/00 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 9 February 2005

Appellant: (Opponent)
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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 18 January 2000 rejecting the opposition filed against the European patent No. 0395736 pursuant to Article 102(2) EPC.

Composition of the Board:

Chairman: L. Galligani
Members: F. L. Davison-Brunel
C. Holtz

Summary of Facts and Submissions

I. European patent No. 0 395 736 with the title "Purified thermostable enzyme" was granted with 18 claims for all designated Contracting States on the basis of the European patent application No. 89 902 422.8.

Granted claims 1 to 3, 6 to 8 read as follows:

"1. A DNA sequence encoding a thermostable DNA polymerase that has the nucleotide sequence encoding the amino acid sequence given in Figure 1.

2. The DNA sequence of claim 1, wherein said polymerase has an amino acid sequence corresponding to

(a) the 832 amino acid sequence of Figure 1;

(b) the amino acid residue 4 to 832 of Figure 1; or

(c) the amino acid residues 290 to 832 of Figure 1.

3. The DNA sequence according to claim 1, wherein up to one third of the 5' coding sequence is absent.

6. The DNA sequence of any one of claims 1 to 5 which encodes a fusion protein containing said DNA polymerase.

7. A DNA sequence encoding a thermostable DNA polymerase which is a modification of the thermostable DNA polymerase having the amino acid sequence referred to in any one of claims 1 to 6, wherein said modification is a deletion, addition, substitution or

another alteration of the amino acids in said amino acid sequence, except for the DNA inserts of recombinant vectors CH35:Taq#4-2 (ATCC 40,336), pFC83(ATCC 67,422), pFC85 (ATCC 67,421) and pLSG1 (composed of the ~750bp BglII/HindIII fragment of pFC83, the ~2,8kbp HindIII/Asp718 fragment of pFC85 and the BglII/Asp718 vector fragment of BSM 13+).

8. A recombinant vector comprising a DNA sequence according to any one of claims 1 to 7, except for the recombinant vector CH35:Taq#4-2 (ATCC 40,336), pFC83(ATCC 67,422), pFC85 (ATCC 67,421) and pLSG1 (composed of the ~750bp BglII/HindIII fragment of pFC83, the ~2,8kbp HindIII/Asp718 fragment of pFC85 and the BglII/Asp718 vector fragment of BSM 13+)."

II. Three oppositions were filed under Article 100(a) EPC, for lack of novelty and inventive step. Two of them were thereafter withdrawn. The opposition division rejected the opposition pursuant to Article 102(2) EPC.

III. The appellant (opponent) filed an appeal, paid the appeal fee and submitted a statement of grounds of appeal together with 17 new documents ((45) to (61)). Arguments against inventive step were raised on the basis of an alleged prior sale of the Taq polymerase enzyme, which had not been reviewed in the decision under appeal. A new ground of appeal was raised, namely Article 100(b) EPC, lack of sufficient disclosure.

IV. The respondent (patentee) answered to the grounds of appeal. A new document (62a-f) was filed.

V. On 20 July 2001, the appellant filed further submissions accompanied by 30 further documents ((63) to (93)).

VI. The board sent a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of appeal, stating its preliminary, non-binding opinion.

VII. This communication was followed by further submissions from both parties, that of the appellant on 7 January 2005 being accompanied by seven further documents ((94) to (100)).

VIII. At oral proceedings which took place on 9 February 2002 the respondent filed a new claim request in replacement of the granted claim request.

Claims 1 and 6 read as follows:

"1. A DNA sequence encoding a thermostable DNA polymerase **consisting of** the nucleotide sequence encoding the amino acid sequence given in Figure 1."(emphasis added by the Board).

"6. The DNA sequence of any one of claims 1 to 5 which **is in fused form and** encodes a fusion protein containing said DNA polymerase." (emphasis added by the board).

Claims 2 to 5, 7 to 18 were identical to the corresponding granted claims. Claims 4 and 5 related to further features of the DNA of claims 1 to 3. Claims 9 to 11 were directed to vectors comprising the DNA sequence of claim 8 and to host cells comprising said

vectors. Claims 12 to 18 related to methods of production or purification of a thermostable DNA polymerase as expressed by the host cells of claims 10 or 11.

IX. The following documents are mentioned in the present decision.

(1): Chien, A. et al., J. of Bacteriology, Vol. 127, No. 3, pages 1550 to 1557, September 1976;

(2): Kaledin, A. S. et al., Translation from Biokhimiya, Vol. 45, No. 4, pages 644 to 651, April 1980;

(5): Lucchini, G. et al., Curr. Genet., Vol. 10, pages 245 to 252, 1985;

(12): Aebersold, R. H. et al., Proc.Natl.Acad.Sci.USA, Vol. 84, pages 6970 to 6974, October 1987;

(27): Alice Jai-Yun Chien Master's thesis, University of Cincinnati, 1976;

(28): David Bruce Edgar, Master's thesis, University of Cincinnati, 1974;

(35): Declaration of Rebecca Kucera with Exhibits 1 to 4 dated 28 February 1998;

(49): Extract from Lab Notebook of Randy Saiki, pages 168 to 172 (27 January 1989));

(51): Extract from an internal Cetus memorandum dated 13 April 1987 summarising assays of NEB Taq;

(52): Page 1257 of the transcript of the US Trial concerning the corresponding case (Gelfand-Direct /Troupis);

(53): Young, R. A. and Davies, R. W., Proc.Natl.Acad.Sci.USA, Vol. 80, pages 1194 to 1198, March 1983;

(56): Kemp, D. J. et al., Molecular Biology of Host-Parasite Interactions, pages 229 to 238, Eds. A. R. Liss, 1984;

(57): Kemp, D. J. et al., Proc.Natl.Acad.Sci.USA, Vol. 80, pages 3787 to 3791, June 1983;

(58): Kemp, D. J. and Cowman, A. F., Proc.Natl.Acad.Sci.USA, Vol. 78, No. 7, pages 4520 to 4524, July 1981;

(64): EP-B-0 258 017 claiming priority dates from 22 August 1986 and 17 June 1987.

X. The appellant's arguments in writing and during oral proceedings which are relevant for the present decision may be summarized as follows:

Admissibility of the documents filed on appeal

The documents relating to the prior sales of Taq polymerase which were filed with the grounds of appeal could not have been filed earlier as they were not

available. Those filed with submissions dated 7 January 2005 had only very recently come to the appellant's attention in the light of concurrent national proceedings in Germany. The other documents also filed on appeal showed that at the priority date, the skilled person would have cloned the Taq polymerase gene without exercising inventive skills. All these documents were prima facie highly relevant and, thus, should be accepted into the proceedings.

Articles 123(2) and 84 EPC; added subject-matter, clarity

- The application as filed did not provide a basis for the subject-matter of claim 1 as it did not disclose a DNA which **consisted in** the nucleotide sequence encoding the amino acid sequence given in Figure 1: the nucleotide sequence depicted in said Figure and the cloned fragments disclosed in the description were all of bigger size than the claimed nucleotide sequence.

- Claims 2 and 3 were internally inconsistent since by way of dependency on claim 1, their subject-matter was the full nucleotide sequence of the Taq polymerase gene whereas, in fact, they related to specific parts of this sequence.
  Claims 4 and 5 had become redundant now that the subject-matter of claim 1 had been restricted to the specific DNA sequence encoding the Taq polymerase protein.

Article 54(3)(4) EPC; novelty; claims 7 and 8

Claims 7 or 8 were not limited to Taq DNA sequences - isolated or as parts of recombinant vectors- only consisting in the DNA encoding the Taq polymerase, as their wording did not exclude the possibility that extra Taq DNA be present upstream or downstream of said sequence. Therefore, they lacked novelty over the disclosure in document (64) of the specific pFC82 recombinant plasmid carrying an 8.0kb HindIII Taq DNA fragment comprising the Taq polymerase encoding DNA.

Article 56 EPC, inventive step; claim 1

- The subject-matter of claim 1 lacked inventive step over the teachings of either of documents (1), (2), (27) or (28) which all disclosed methods for the purification of the Taq polymerase enzyme. Their technical teaching had already been extensively discussed in the appeal case T 1080/01 of 24 October 2003 dealing with the European patent which was document (64) in the present proceedings. The then competent board concluded that they did not enable the isolation of the enzyme and thus, decided in favour of inventive step of a cloned DNA expressing it. Yet, these findings were not binding on the present board and, thus, the appellant maintained all his arguments against inventive step on the basis of said documents.

- The subject-matter of claim 1 also lacked inventive step in view of the prior sales of the Taq polymerase enzyme by New England Biolabs (NEB)

which had occurred prior to the priority date of the patent in suit (12 January 1988). In fact, seven lots of it had been sold up until that date, as was shown in Exhibits 3 and 4 which accompanied document (35). The enzyme was free of contaminating proteins such as nucleases (document (49), page 170). Its purity was even acknowledged by the respondent during the US trial (document (52)).

A pure preparation of the Taq polymerase enzyme being available, it was a matter of routine to isolate the gene by the technique of expression cloning using λgt11 as cloning vector (documents (53), (56), (57), (58)). To obtain antibodies against said enzyme - ultimately to be used for screening the positive clones expressing it- was, as admitted by the respondent, an easy if somewhat lengthy task.

If the commercially available preparation was not considered sufficiently pure for raising specific anti-Taq polymerase antibodies, it could be purified further according to well-known methods such as separation from contaminating moieties by "activity gels". It should also be kept in mind that retrieving a positive clone was possible even if the antibodies used for screening were not all directed against the Taq polymerase, as it was just a matter of testing more of the prima facie positive clones for their ability to express the enzyme (parts thereof).

Even a fragment of the Taq protein could be used for raising antibodies suitable for screening. Alternatively, antibodies against a related

protein such as E.coli DNA polymerase I would also have been suitable.

The isolation of the Taq polymerase gene would also be achieved in an obvious manner by screening the Taq DNA library with multiple degenerated oligonucleotide probes. The relevant oligonucleotide sequences would easily be determined after the commercially available Taq polymerase enzyme had partially been sequenced.

For these reasons, the subject-matter of claim 1 was obvious.

XI. The respondent's arguments in writing and during oral proceedings which are relevant for the present decision may be summarized as follows:

Admissibility of the documents filed on appeal

The filing of evidence regarding prior sales one month before the oral proceedings was so late as to make it impossible for the respondent to check whether or not this evidence was factually correct. Accordingly, it should be dismissed in its entirety. The respondent was not aware of any national proceedings in Germany in which the belatedly submitted evidence had been cited. Alternatively, the oral proceedings should be postponed until the second half of the year 2005.

Articles 123(2) and 84 EPC; added subject-matter, clarity

- Support for the subject-matter of claim 1 was found in the application as filed page 3, lines 21 to 23 as well as in Figure 1. Support for the subject-matter of claim 6 was found on page 12, lines 30 to 31 and page 13, lines 29 to 33.

- In accordance with the case law, the patent should be read with the mind of a skilled person willing to understand. There was no doubt that claims 2 and 3 made technical sense since the parts of the Taq DNA to which they related were clearly identified. As for claims 4 and 5, it was at a patentee's discretion to draft dependent claims to specific features which were implicit in the wording of the claim they depended upon.

Article 54(3)(4) EPC, novelty; claims 7 and 8

Neither of claims 7 or 8 could be construed as being directed to a Taq DNA sequence of bigger size than that consisting of the nucleotide sequence encoding the amino acid sequence given in Figure 1, since both these claims were dependent on claim 1 (relating to that nucleotide sequence) or to claims 2 to 6 which related to DNA sequences comprising Taq DNA of the same size or smaller than that in claim 1. Thus, document (64) which disclosed a recombinant plasmid pFC82 carrying 8.0kb of Taq DNA itself containing the 2.45kb Taq polymerase gene was not detrimental to novelty.

Article 56 EPC, inventive step; claim 1

- The technical contents of documents (1), (2), (27) or (28) had already been amply discussed in the appeal case T 1080/01 (supra) and there was no reasons why the board in its present composition should depart from the conclusion reached by the then competent board that none of them provided such an enabling disclosure of the natural Taq polymerase that the enzyme could be used as the starting material to clone the Taq polymerase gene in an obvious manner.

- The alleged prior sales of Taq polymerase were not relevant to inventive step for two reasons: first, they had not been proven up to the hilt as was necessary in accordance with the case law. Second, even if it was accepted that prior sales had taken place, the enzyme which had been sold was not in such a pure state as to enable the production of an antibody preparation suitable for screening in an expression cloning experiment. The fact that the enzyme was active did not necessarily imply that it was pure. Document (49) did not indicate the degree of purity (only that it did not contain nucleases). As for document (52), it provided no evidence that the NEB enzyme then mentioned by the respondent had ever been sold.
  In contrast, a successful cloning experiment requested a preparation of highly specific antibodies which could only be obtained using a highly purified preparation of the polymerase. For this reason, the availability of commercial Taq polymerase was not at all sufficient to render

obvious the isolation of the Taq polymerase gene by expression cloning.
The appellant's argument that if the enzyme preparation was thought not to be pure, it could always be purified by routine procedure before raising antibodies against it had been found not convincing in T 1080/01 (supra) and there was no reasons to depart from this earlier finding. As for the use of antibodies raised against E.coli DNA polymerase to screen the clones expressing Taq polymerase, this could not have been envisaged at the priority date since no sequence relationship was known to exist between the two enzymes.

- The sequencing of the commercially available Taq polymerase, with the view of ultimately producing multiple degenerated oligonucleotide probes could not be put into practice even if the enzyme was highly purified (which it was not) since its N-terminal end was blocked.

For all these reasons, inventive step must be acknowledged.

XII. The appellant requested that the decision under appeal be set aside and that the European patent No. 0 395 736 be revoked.

The respondent requested that the decision under appeal be set aside and the patent be maintained with claims 1 to 18 as filed in the oral proceedings.

Reasons for the decision

Admissibility of the documents filed on appeal

1. The appellant filed 17 documents with the grounds of appeal, 30 further documents with the submission dated 20 July 2001 and, there again, 7 more documents one month before the oral proceedings.

2. The last seven documents ((94) to (100)) were filed as additional evidence in relation to the prior sales of the NEB enzyme. The issue of prior sale was already mentioned in the minutes of the oral proceedings before the opposition division (February 1999). It was of ominous importance in the case T 1081/01 (supra) relating, in particular, to the natural Taq DNA polymerase and involving the same parties. In view of the decision then rendered, it could not have escaped the appellant that it would also be a key issue in the present appeal. In the board's judgment, all evidence relating to prior sales should have been sought for, found and submitted well ahead of the present oral proceedings. The documents are rejected for being late filed.

3. Of the 30 documents filed with the submissions dated 20 July 2001 ((63) to (93)), some bring technical evidence relating to the Taq polymerase. The remainder describe prior art information relating to DNA polymerases in general, to methods of protein purification, identification or sequencing, to methods for cloning DNA. Prima facie, these documents do contain interesting but not essential information taking into account the information already on file.

Consequently, the Board uses its discretion under Article 114(2) EPC to disregard them.

4. The 17 documents filed with the grounds of appeal ((45) to 61)) are accepted into the proceedings. Document (62a-f) filed by the respondent in its reply is also admitted.

Formal requirements

Article 123 EPC; claims 1 and 6

5. Claims 1 and 6 have been amended on appeal (section VIII supra). A basis for the amended subject-matter of claim 1 can be found in the application as filed, pages 3, lines 21 to 23 and 4, lines 24 to 27:

"Figure 1 is the DNA sequence and the predicted amino acid sequence for Taq polymerase. The amino acid sequence corresponding to the deduced translation product is numbered 1-832."

"In one embodiment of the invention, the DNA sequence encoding a full-length thermostable DNA polymerase of Thermus aquaticus (Taq) is provided. Figure 1 shows this DNA sequence and the deduced amino acid sequence.",

together with Figure 1. Admittedly, the whole depicted sequence comprises 120 bp upstream and 10 bp downstream from the Taq polymerase coding sequence per se. Yet, this sequence is unambiguously identified as from bp 1 to bp 2496. A basis thus exists for acknowledging that

the requirements of Article 123(2) EPC are fulfilled by claim 1.

6. The same conclusion is reached in relation to the subject-matter of claim 6 which finds a basis, in particular, on page 13, lines 29 to 34 of the application as filed:

"First, a DNA is obtained that encodes ... a fusion of the Taq polymerase to an additional sequence ..."

7. As the subject-matter of claim 1 has been limited compared to the subject-matter of granted claim 1 (section I, supra), and all further claims are directly or indirectly dependent on claim 1, the requirements of Article 123(3) EPC also are fulfilled.

Article 84 EPC; clarity

8. Claims 1 and 6 were not argued against for lack of clarity. The board also considers them to be clear.

9. In accordance with the case law (T 190/99 of 6 March 2001) a patent must be construed by a mind willing to understand and not by a mind desirous of misunderstanding. Following this approach, the board finds that the subject-matter of claims 2 and 3 has not become unclear by becoming dependent on amended claim 1 because the claimed specific sequences are unambiguously defined.

10. As for the subject-matter of claims 4 and 5 - dependent on claim 1 -, it is true that it is implicitly

contained in said claim. It being now explicitly disclosed can only make it clearer.

11. The requirements of Article 84 EPC are fulfilled.

Article 54(3)(4) EPC, novelty; claims 7 and 8

12. Claim 7 (section I, supra) relates to a DNA sequence encoding a modified Taq polymerase compared to that referred to in claim 1. Claim 1, in turn, relates to the DNA consisting of the nucleotide sequence encoding the enzyme (2496 bp, section VIII, supra) i.e. containing no extra DNA upstream or downstream of said sequence. The modifications are all to take place within the amino acid sequence of the enzyme i.e. they are due to alterations **within** the DNA of claim 1. Claim 6, thus, relates to a DNA consisting of an altered nucleotide sequence which, like the DNA of claim 1, contains no extra DNA upstream or downstream of said sequence.

13. Document (64), which is a prior art document pursuant to Article 54(3)(4) EPC (section IX supra), discloses on page 29 the recombinant plasmid pFC82 comprising 8.0kb of Taq DNA from which Taq polymerase is expressed. In the board's judgment, the 8.0kb Taq DNA fragment is not detrimental to the novelty of the DNA of claim 7 if only because it contains extra DNA on either size of the Taq polymerase coding sequence.

14. Claim 8 relates to a recombinant vector comprising a DNA sequence according to any one of claims 1 to 7, i.e. maximally consisting of the Taq polymerase coding sequence (internally altered or not). The appellant's

argument that there might be more Taq DNA in the vector than this sequence per se only makes sense if the expression "A recombinant vector **comprising** ..." is interpreted as meaning that the recombinant vector comprises a vector part, the explicitly mentioned Taq polymerase sequence and, in addition, some unspecified Taq DNA. To the board, this interpretation goes against that which the mind willing to understand would adopt, namely that the recombinant vector comprises a vector part and a recombinant part, the latter being the explicitly identified "foreign" DNA - in this case the DNA according to any one of claims 1 to 7 of maximally 2496 bp. For this reason, it is concluded that the recombinant vector pFC82 which comprises a vector part and 8.0kb of "foreign" Taq DNA does not destroy the novelty of the subject-matter of claim 8.

Article 56 EPC, inventive step; claim 1

15. Two approaches were taken to inventive step on the basis either of the prior art documents (2), (1) or (27), and (28) or of the prior sales of Taq polymerase. The rationale behind them was the same: that the provision of methods for purifying the enzyme or, of the enzyme per se made obvious the subsequent cloning of the Taq polymerase gene.

16. Documents (2), (1) or (27), and (28) respectively correspond to documents (13), (9) or (10), and (3) in the earlier appeal case T 1080/01 (supra). This case related to the European patent identified in the present proceedings as document (64) - a piece of prior art which may not be taken into account for the assessment of inventive step (see point 13, supra)-

which discloses natural Taq polymerase and recombinant clones expressing said polymerase. The same parties were involved in both cases. The technical content of documents (2), (1) or (27), and (28) was extensively investigated by the then competent board (points 45 to 66 of the decision) who came to the conclusions that document (13) did not disclose a method leading to the purification of the Taq polymerase and that the experimental evidence provided to show that the protein isolated by the methods described in documents (3), (9) or (10) was Taq polymerase was at best inconclusive.

17. The present board carefully reviewed the four prior art documents and came to the same conclusion as in T 1080/01 as regard their contents. At oral proceedings, the appellant provided no further arguments regarding the suitability of the methods therein described for the isolation of Taq polymerase. Accordingly, it is concluded that neither of the documents enables the purification of the native Taq polymerase. As one of the essential material for cloning the Taq polymerase gene is the Taq polymerase enzyme irrespective of the cloning method used, documents (2), (1) or (27), and (28) are not relevant for the assessment of inventive step.

18. Inventive step of the claimed DNA starting from the prior sales of Taq polymerase is not one of the issue reviewed in the decision under appeal. Yet, according to the Minutes of the oral proceedings before the opposition division (point 2.2, fourth full paragraph), it was an issue argued (against) by the present appellant (then opponent). He had then requested that a document be introduced in the proceedings which, in his

view, constituted evidence of prior sale. This request was refused for being late filed. It is clear from the Minutes (point 2.6) that the opposition division investigated the prima facie relevance of the document and found it too ambiguous to be of use. Thus, it appears that the issue was taken into consideration to the extent possible.

19. None of the parties in the present appeal expressed any objections to inventive step vis-à-vis the prior sale product being dealt with by the board. Considering that the patent in suit was filed some sixteen years ago, the board decides to assess the matter.

20. The prior sales of Taq polymerase by NEB is acknowledged in the patent in suit (page 2, lines 26 to 28). Exhibits 3 and 4 accompanying document (35) show that seven lots of the enzyme were sold before the priority date (12 January 1988). Each of them had been prepared by a different purification method (Exhibit 3). Their purity was analysed on SDS-polyacrylamide gel (Exhibit 4). At oral proceedings the appellant confirmed that these lots were sold, which the respondent did not deny, except for Lot 1 which was the subject-matter of much controversy all through the written proceedings. There remains, however, the fact that, as indicated in the patent in suit, Taq polymerase was sold before 12 January 1988.

21. Starting from the prior sales of the Taq polymerase, the problem to be solved can be defined as obtaining the DNA encoding said enzyme.

22. The solution to this problem is given in claim 1 which relates to the DNA encoding Taq polymerase as defined by its sequence.

23. The question which must be answered is, thus, whether or not at the priority date, it would have been obvious to clone this DNA, - ie the skilled person would have had a reasonable expectation of success starting from a commercial preparation of Taq polymerase. Two approaches are said to have been available: expression cloning and cloning with using multiply degenerate oligonucleotide probes for screening purposes.

24. Expression cloning is a method which requires that the positive recombinant clones amongst a λgt11 library be identified as such by their ability to produce the Taq polymerase (fragments thereof) i.e. by their ability to fix antibodies raised against the enzyme. A number of documents were cited to illustrate this last point. Two of them (documents (56) and (57)) are not relevant insofar as they relate to the simultaneous isolation of multiple genes rather than to the isolation of one gene. In document (53), it is mentioned on page 1197, right-hand column:

"Antibody quality plays an important role in a successful screen...The best specific reaction with antigens produced by λgt11 recombinants should be obtained with affinity purified antibodies."

In document (58) relating to the detection of clones that contain polypeptides encoded by cloned DNA segments, the antiserum used is also affinity purified (page 4521, left-hand column). In document (5) the

antibodies - to be used for detecting clones expressing yeast DNA polymerase I - are raised against a preparation of the enzyme which is purified to near homogeneity (page 246, right-hand column). All these prior art teachings go towards the following conclusion: the specificity of the antibody preparation plays a key role for the successful screening of the positive clones with the corollary that a very pure protein must be available.

25. The purity of the commercially available Taq polymerase was essentially argued by the appellant on the basis of documents (49), (51) and (52). Document (49) (page 170) is a data sheet relating to the commercial enzyme which informs the customer that it is free of nucleases ("without any detectable degradation of the DNA"). This is not evidence that it is free of other contaminating moieties. Document (51) shows that PCR technology was carried out with the NEB enzyme as early as 1987. Whereas this implies that the enzyme was active, it is not informative at all as to its state of purity. Document (52) is a value judgment of the respondent in the US trial to the avail that NEB enzyme as provided to him "was very nice", "of a comparable degree of purity that we had obtained". Whether this degree of purity is compatible with raising the antibody preparation suitable for screening in an expression cloning experiment is, as would be expected, not specified.

26. In fact, as already above mentioned (point 20, supra), there exist experimental data on file concerning purity submitted by the appellant, namely Exhibit 4 of document (35). Leaving aside for the present purpose

any consideration of the "disputed" Lot 1, it is readily apparent from these data that, whereas Lots 2 and 3 do not seem to contain any proteins other than the Taq polymerase and BSA (Lot 3), Lots 4 to 6 respectively contain at least 6, 1 and at least 8 contaminating proteins. As for Lot 7, it seemingly contains no Taq polymerase but a host of other proteins, which strongly suggests that the polymerase is a minor protein species in the sample. This has dire consequences: it means that depending on the point in time prior to the priority date when the skilled person would buy the enzyme, he/she would be handling preparations of widely diverging states of purity. If as required by the expression cloning, he/she is to use any of them to raise antibodies against, his/her chance of obtaining an antibody preparation suitable for screening and, therefore, to isolate the DNA encoding Taq polymerase is purely a matter of luck. Otherwise stated, his/her expectation of success irrationally ranges from nil to high and this unfortunate situation cannot be regarded as a **reasonable** expectation of success (see in this context, T 737/96 of 9 March 2000).

27. The appellant further argued that the enzyme, if considered not to be pure enough, could always be further purified by well-tried techniques. This may well be true but, in the board's judgment, it represents what the skilled person could do with the technology available at the time rather than what he/she would attempt to do with a reasonable expectation of success, furthermore keeping in mind that, due to their size, commercial preparations are intended for analytical rather than preparative purposes. The suggestion was also made that antibodies

could be raised against fragments of the enzyme rather than the whole molecule, but this, of course, requires that the protein is purified in the first place. As for examining more clones with a less pure antibody preparation, there again, it amounts to relying on luck and, as just mentioned, luck is no indicia of a reasonable expectation of success. Finally, the appellant argued that antibodies raised against a further DNA polymerase, such as E.coli DNA polymerase I could have been used. However, there is no evidence on file that at the priority date (and, may be, up until the present time), cross-reactivity of anti-DNA polymerase I antibodies with Taq DNA polymerase was established.

28. The second cloning approach which was cited, ie the use of multiple degenerated probes for screening a Taq DNA library is also flawed for essentially the same reasons: to devise these probes, it would have been necessary to have some information on the sequence of the Taq polymerase enzyme and this, in turn, would have required that a highly purified enzyme preparation be available for amino acid sequencing. Even if it were available, this would not guarantee that the sequence would be obtained as the N-terminal end of the protein would seem to be blocked. The counterargument was presented that at the priority date, a technique had already been described which enabled the sequencing of internal fragments of a protein (document (12)). As in point 27 above, the board remarks that this is evidence as to what the skilled person could have done in one of the steps preceding the screening of the library, but not evidence that the skilled person would attempt to

achieve the isolation of the relevant recombinant clones with a reasonable expectation of success.

29. For these reasons, the board concludes that claim 1 to the DNA encoding the Taq polymerase enzyme enjoys inventive step as well as all further claims which are dependent on said claim.

Admissibility of a new ground of opposition

Article 83 EPC; sufficiency of disclosure

30. Article 100(b) was only introduced as a ground of opposition at the appeal stage (grounds for the appeal, page 10 onwards). With his submission dated 21 December 2004, the respondent requested that the new ground of opposition be rejected. In accordance with the Enlarged Board of Appeal opinion G 10/91 (supra, point 3. of the opinion), "Fresh grounds of opposition may be considered in appeal proceedings only with the approval of the patentee." Following this case law, the board will not consider sufficiency of disclosure.

31. For the above mentioned reasons, it is concluded that the claim request filed at oral proceedings on 9 February 2005 fulfils the requirements for patentability.

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 with claims 1 to 18 as filed in the oral proceedings and description and figures as granted.

The Registrar: The Chairman:

A. Wolinski L. Galligani