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D E C I S I O N
of 24 October 2003

Case Number: T 1080/01 - 3.3.8

Application Number: 87307433.0

Publication Number: 0258017

IPC: C12N 15/10

Language of the proceedings: EN

Title of invention:
Purified thermostable enzyme and process for amplifying, detecting, and/or cloning nucleic acid sequences using said enzyme

Patentee:
F. HOFFMANN-LA ROCHE AG

Opponents:
New England Biolabs Inc.
Bioline (UK) Ltd
Promega Corporation
Becton, Dickinson and Company

Headword:
Thermostable enzyme/F. HOFFMANN-LA ROCHE

Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 87, 104(1), 114(1), 123(2)
EPC R. 23c, 57a

Keyword:
"Main request: allowability of amendments (no)"
"Auxiliary request: allowability of amendments (yes)"
"Entitlement of claim 1 to the earlier priority date (yes)"
"Clarity (yes)"
"Sufficiency of disclosure (yes)"
"Novelty (yes)"
"Inventive step (yes)"
"Apportionment of costs (no)"

Decisions cited:
G 0002/98, T 0060/89, T 0397/89, T 0606/89, T 0666/89, T 0157/90, T 0432/92, T 0465/92, T 0793/93, T 0493/94, T 0750/94, T 0848/94, T 0950/99, T 0670/00, T 0397/02

Catchword:
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Case Number: T 1080/01 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 24 October 2003

Appellant: (Proprietor of the patent)
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Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted 30 August 2001 revoking European patent No. 0258017 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: F. L. Davison-Brunel
Members: T. J. H. Mennessier
V. Di Cerbo

Summary of Facts and Submissions

I. European patent 0 258 017 with the title "*Purified thermostable enzyme and process for amplifying, detecting, and/or cloning nucleic acid sequences using said enzyme*" was granted on European patent application No. 87307433.0 claiming priority from four American patent applications (US 899513 and US 899241, both filed on 22 August 1986, as well as US 63647 and US 63509, both filed on 17 June 1987).

Claims 1 to 4 as filed read as follows:

"1. A purified thermostable enzyme that catalyzes combination of nucleotide triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand."

"2. An enzyme according to claim 1 that is DNA polymerase."

"3. An enzyme according to claim 1 or 2 that has a molecular weight of about 86,000 to 90,000 daltons."

"4. An enzyme according to claim 3 from **Thermus aquaticus**."

Granted claim 30 read as follows:

"30. A stable enzyme composition comprising a thermostable enzyme having DNA polymerase activity of any one of claims 1 to 8, a recombinant thermostable enzyme or fragment thereof having DNA polymerase activity obtained by the method of claim 24 or 25, or a

recombinant enzyme or modification thereof having DNA polymerase activity of any one of claims 26 to 29 in a buffer comprising one or more non-ionic polymeric detergents."

II. The patent was then opposed by four parties (opponents 1 to 4, now respectively respondents I to IV) on the grounds as set forth in Articles 100(a), (b) and (c) EPC that the invention was not new (Article 54 EPC), did not involve an inventive step (Article 56 EPC), was not sufficiently disclosed (Article 83 EPC), and that the patent contained subject-matter extending beyond the content of the application as filed (Article 123(2) EPC).

III. The opposition division revoked the patent by a decision given at oral proceedings on 28 May 2001, with written reasons posted on 30 August 2001. Basis for this decision were the granted claims, taken as the main request, as well as four auxiliary requests (I to IV) filed on 28 May 2001. The reasons for the revocation were: (i) presence of added matter in claims 1 and 26 of the main request, (ii) extension of the scope of claim 1 of auxiliary request I compared to granted claim 1, (iii) lack of novelty of claims 1 and 26 of auxiliary request II and of claim 26 of auxiliary request III, and (iv) lack of inventive step of claims 1 to 32 of auxiliary request IV.

IV. The patentee (appellant) lodged an appeal against the decision of the opposition division. A statement setting out the grounds of appeal was filed on 9 January 2002, accompanied by a new main request for all designated Contracting States except AT and ES and

corresponding claims for the designated Contracting States AT and ES. Accelerated handling of the case was requested.

The main request for all designated Contracting States except AT and ES consisted of 37 claims. Claims 1 and 26 read as follows:

"1. A thermostable enzyme having DNA polymerase activity that catalyses the combination of nucleoside triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand, that has a molecular weight of 86,000 to 90,000 as determined according to its migration in SDS-PAGE, when the marker proteins are phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500) and lysozyme (14,400)."

"26. A recombinant thermostable enzyme having DNA polymerase activity or a modification thereof having DNA polymerase activity that catalyzes the combination of nucleoside triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand, said enzyme or modification thereof having a molecular weight of 86,000 to 90,000 as determined according to its migration in SDS-PAGE, when the marker proteins are phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500) and lysozyme (14,400), said enzyme being obtainable by the method of claim 24 or 25, wherein said host cell is the recombinant host cell of claim 23."

V. Respondents II and III filed observations in reply to the statement of grounds of appeal.

VI. A communication under Article 11(2) of the Rules of Procedure of the Boards of Appeal presenting some of the board's preliminary and non-binding views was sent to the parties together with the summons to oral proceedings.

VII. On 29 August 2003, in reply to the board's communication, the appellant filed further observations together with 43 additional documents.

VIII. Respondents I, II and III objected to the introduction of these documents into the appeal proceedings and requested that oral proceedings be postponed or that costs be apportioned in case the oral proceedings were maintained and the board decided to admit the documents.

IX. With a communication dated 13 October 2003, the board informed the parties that the oral proceedings were maintained as scheduled and that the admissibility of the documents filed on 29 August 2003 would then be discussed.

X. At the oral proceedings which took place on 22, 23 and 24 October 2003, the appellant filed an auxiliary request for all designated Contracting States except AT and ES, as well as corresponding claims (1 to 51) for the Contracting States AT and ES.

The auxiliary request (auxiliary request I) for all designated Contracting States except AT and ES

consisted of 28 claims. Claims 1, 5, 16, 18, 21 and 27 read as follows:

"1. A thermostable **Thermus aquaticus** DNA polymerase that catalyses the combination of nucleoside triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand, that has a molecular weight of 86,000 to 90,000 as determined according to its migration in SDS-PAGE, when the marker proteins are phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500) and lysozyme (14,400)." (emphasis added by the board)

"5. A DNA sequence encoding a thermostable **Thermus aquaticus** DNA polymerase that catalyses the combination of nucleoside triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand according to any one of claims 1 to 4 or **a fragment of** said DNA sequence encoding an enzymatically active, truncated thermostable enzyme having DNA polymerase activity." (emphasis added by the board)

"16. A method for the production of a recombinant thermostable enzyme **having DNA polymerase activity** or **fragment thereof** having DNA polymerase activity, which catalyses the combination of nucleoside triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand, said method comprising the culturing of a host cell of claim 14 or 15." (emphasis added by the board)

"18. A recombinant thermostable enzyme having DNA polymerase activity or **a modification thereof having DNA polymerase activity** that catalyzes the combination of nucleoside triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand, said enzyme or modification thereof having a molecular weight of 86,000 to 90,000 as determined according to its migration in SDS-PAGE, when the marker proteins are phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500) and lysozyme (14,400), said enzyme being obtainable by the method of claim 16 or 17, wherein said host cell is the recombinant host cell of claim 15." (emphasis added by the board)

"21. A stable enzyme composition comprising a recombinant thermostable Thermus aquaticus DNA polymerase or **a modification thereof** having DNA polymerase activity that catalyses the combination of nucleoside triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand, that has a molecular weight of 86,000 to 90,000 as determined according to its migration in SDS-PAGE, when the marker proteins are phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500) and lysozyme (14,400) in a buffer comprising one or more non-ionic polymeric detergents." (emphasis added by the board)

"27. The use of a thermostable enzyme **having DNA polymerase activity** of any one of claims 1 to 4, of the recombinant thermostable enzyme of claim 18 or of a

stable enzyme composition of any one of claims 19 to 21 or 24 to 26 for polymerase chain reactions."

Dependent claims 2 to 4 related to further features of the DNA polymerase of claim 1. Claims 6 to 10 related to further features of the DNA sequence of claim 5. Claims 11 to 15 respectively related to recombinant vectors containing the DNA sequence of claims 5 to 10 or to host cells containing such a vector. Claim 17 related to a further feature of the method of claim 16. Claims 19, 20 and 22 to 26 related to various compositions comprising a Thermus aquaticus DNA polymerase, in recombinant form or not, having the features of the enzyme of claim 1 in a buffer comprising one or more non-ionic polymeric detergents. Claim 28 related to a method for the amplification of nucleic acid sequences comprising the use of the thermostable DNA polymerase as previously claimed.

XI. The following documents are mentioned in the present decision:

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

(9): Alice Chien et al., J. Bacteriol., Vol. 127, No. 3, September 1976, Pages 1550 to 1557;

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

(13): English translation of "A. S. Kaledin et al., Biokhimiya, Vol. 45, No. 4, April 1980, Pages 644 to 651", Ed. Plenum Publishing Corporation, 1980, Pages 494 to 501;

(17): David Freifelder, Physical Biochemistry, Applications to Biochemistry and Molecular Biology, Second Edition, W. H. Freeman and Company, San Francisco, 1982, Pages 270 and 271

(23): Leszek J. Klimczak et al., Nucleic Acids Res., Vol. 13, No. 14, 1985, Pages 5269 to 5282;

(28): Leszek J. Klimczak et al., Biochemistry, Vol. 25, 1986, Pages 4850 to 4855;

(30): Matthew J. Longley et al., Nucleic Acids Res., Vol. 18, No. 24, 1990, Pages 7317 to 7322;

(34): Youngsoo Kim et al., Nature, Vol. 376, 17 August 1995, Pages 612 to 616;

(37): Declaration of Alex Kaledin dated 2 March 1998;

(40): Declaration of Diane Rein dated 2 March 1998;

(43): Declaration of Leszek Janusz Klimczak dated 27 February 1998;

(44): Declaration of Randall Dimond dated 3 March 1998;

(47): Declaration of Rebecca Kucera dated 28 February 1998;

(61): Declaration of Michael J. Chamberlin dated 6 February 1997 and annexed Exhibits E and F;

(76): Lewin R., Science, Vol. 233, No. 4760, 11 July 1986, Page 159;

(78): Seven documents headed "invoice" from "New England Biolabs" with shipping dates of February and March 1997;

(79): One page paper from "New England Biolabs" providing information with respect to an assay dated 20 March 1987 concerning "#252 / T.aquaticus DNA polymerase Lot# 1";

(80): Laboratory notebook of S. Stoffel (Pages 184 to 187);

(81): Laboratory notebook of R. Saiki (Pages 168 to 172);

(85): Richard A. Young and Ronald W. Davis, Proc. Natl. Acad. Sci. USA, Vol. 80, 1983, Pages 1194 to 1198;

(93): Bruno Oesch et al., Cell, Vol. 40, 1985, Pages 735 to 748;

(110): Randall L. Dimond and William F. Loomis, J. Biol. Chem., Vol. 251, No. 9, 1976, Pages 2680 to 2687;

(112): Ad Spanos et al., Nucleic Acid Res., Vol. 9, No. 8, 1981, Pages 1825 to 1839;

(200): EP 0 201 184 B1;

(218): A. Blank et al., Anal. Biochem., Vol. 135, 1983, Pages 423 to 430;

(230): Laboratory notebook of Diane Rein received at the EPO on 9 January 2002;

(244): Declaration of Randall L.Dimond dated 19 February 2001;

(273): Declaration of Donald A. Cowan dated 9 December 1994;

(276a): Frances C. Lawyer et al., PCR Methods and Applications, 1993, Pages 275 to 287;

(281): Declaration of Alexander Grigorevich Slyusarenko dated 24 May 1994.

XII. The appellant's arguments in writing and during oral proceedings, insofar as they are relevant to the present decision, may be summarised as follows:

Admissibility of the 43 documents filed on 29 August 2003; requests for reimbursement of costs

These documents were not filed at the very end of the one month period before the oral proceedings but seven weeks before the oral proceedings in order to allow everybody involved more time to study them. They were already known by the respondents since they had earlier been filed in the proceedings before other courts.

Their present filing was necessary to counter respondent III's allegations which had been made at the oral proceedings before the opposition division, especially since during these earlier oral proceedings respondent III had objected to them being referred to on the ground that they had not yet been filed at the EPO.

The respondents' requests for reimbursement of costs had no basis because it was in the nature of opposition proceedings that the representatives might have to study the file intensively more than just once.

Main request for all designated Contracting States except AT and ES

Rule 57a EPC

Claim 26 (see section IV, supra) related to the Taq DNA polymerase as obtained by a recombinant process. It was introduced in an attempt to overcome the objection of lack of novelty raised on the basis of prior art documents disclosing a DNA polymerase directly obtained from Thermus aquaticus (also identified thereafter as T. aquaticus).

Claims 27 to 32 related to independent embodiments which were all present in granted claim 30. The claimed subject-matter had been so rearranged in order to facilitate the drafting of auxiliary requests, if needed.

Article 123(2) EPC; added matter; claim 1

In accordance with the case law, originally filed claims were part of the originally filed disclosure. In claim 3 as filed, the claimed DNA polymerase was defined amongst other features by its molecular weight, it being recited without a further definition of a method and of the marker proteins used for its determination. There could be no doubt that the skilled person would logically deduce from the application as filed (see, in particular, page 3, lines 1 to 13, and page 14, line 32 to page 16, line 11) that this molecular weight was to be determined by the method and with the help of the marker proteins used for the determination of the molecular weight of the T. aquaticus DNA polymerase and, thus, corresponded to the definition given in claim 1 of the main request. This was especially true since no other method for the determination of molecular weight had been mentioned.

Alternatively, a basis could be found for the subject-matter of claim 1 in the patent application *per se* on page 2, lines 41 and 42 (pointing to a thermostable enzyme with nucleotide triphosphates polymerising activity) together with page 6, lines 16 to 21 (indicating many sources for the enzyme), page 7, lines 8 to 11 (where the molecular weight was said to be determined by SDS-PAGE using protein markers) and Example I, page 23, lines 48 to 50 (defining these markers in relation to T. aquaticus DNA polymerase)(the page numbers refer to the A2-publication).

The case was alike that dealt with in decision T 493/94 of 4 August 1999 where the board decided that the requirements of Article 123(2) EPC were fulfilled in relation to an individual hG-CSF species defined by a specific activity value whereas this specific activity had been determined for a mixture of three forms of the molecule differing by their isoelectric points. The board concluded (see point 6c of the reasons of the decision) that *"what matters are the contents of the application as filed and what the skilled person would logically deduce therefrom."*. The only deduction to be drawn from the originally filed application in the present case was that the subject-matter of claim 3 as filed corresponded to that of claim 1. The requirements of Article 123(2) EPC were fulfilled.

Auxiliary request for all designated Contracting States except AT and ES

Article 123(2) EPC; added matter; claim 1

It was clear from the application as filed that the Thermus aquaticus strain used as a source of the claimed enzyme needed not be YT1 as on page 7 (A2-publication) mention of the T. aquaticus enzyme was made several times without specifying the strain it came from. Claiming T. aquaticus DNA polymerases in general did not offend the requirements of Article 123(2) EPC.

Article 84 EPC; clarity

The auxiliary request had been limited to the Thermus aquaticus polymerase/polymerase encoding gene and,

therefore, no ambiguity resulted from the origin of said enzyme/DNA being mentioned in some of the claims only by way of dependency.

The terms "*fragment of/thereof*" and "*modification thereof*" relating to the Taq polymerase DNA or Taq polymerase enzyme were already present in the granted claims. No objections of lack of clarity could be raised against them.

Article 83 EPC; sufficiency of disclosure in relation to the Taq DNA polymerase

The specific experimental conditions in which to run the SDS-PAGE in order to determine the molecular weight of the DNA polymerase needed not be disclosed as the skilled person would be well aware of them. Document (61) showed that by the time of the invention, SDS-PAGE electrophoresis was one of the most common and routine techniques in biochemistry laboratories worldwide and referred to two standard publications made by Laemmli and Weber et al. which were common knowledge at the priority date. Using the teaching of said publications, the skilled person would have been in a position to carry out a SDS-PAGE to determine the molecular weight of the DNA polymerase obtainable from Thermus aquaticus.

Article 87 EPC; entitlement to priority

Whereas the enzyme and use thereof were entitled to the earlier priority date (22 August 1986), all the other claimed aspects of the invention (DNA sequence, recombinant enzyme, stable enzyme composition and uses

thereof) were entitled to the latter priority date (17 June 1987). Example I in the earlier priority documents US 899513 and US 899241 described a method for the purification of the claimed natural enzyme. Therefore, even in the absence in both documents of an example equivalent to Example VI of the patent, the enzyme was entitled to the earlier priority date.

Article 54 EPC; novelty; claims 1 and 18

- Claim 1

The enzyme which was the gist of the invention was the full-length DNA polymerase obtainable from Thermus aquaticus, also known as the **Taq [DNA] polymerase**, ie the enzyme as represented on Figure 2 of document (34), having 5' to 3' exonuclease activity with an apparent molecular weight of 92,000 daltons as determined by SDS-PAGE (see document (30)).

In contrast, the "Trela group" of research workers including the authors of documents (3), (9) and (10), had purified and characterised a DNA polymerase of smaller molecular weight (document (3): 72,000 daltons as determined on sucrose gradient; documents (9) and (10): 63,000 daltons as determined by gel filtration and 68,000 daltons as determined on sucrose gradient). This was probably because the purification had been performed at room temperature and proteolysis had occurred; molecular entities different from the native full-length Taq DNA polymerase had been obtained, namely fragments thereof. A further evidence that the enzyme described in document (9) was not the native full-length Taq DNA polymerase could be found in the

fact that it did not exhibit 5' to 3' exonuclease activity (see abstract and page 1552).

Document (13) also disclosed a DNA polymerase without nuclease activity (see page 500). Its molecular weight as determined by SDS-PAGE under denaturing conditions was small (62,000 daltons). One of the co-authors of document (13) declared that the enzyme was not the full-length polymerase (see document (281)).

The respondents attempted to show that the full-length enzyme was known at the priority date by reproducing the purification methods described in either of the previously mentioned documents. These attempts all failed because the protocols which had been used always differed from the earlier protocols in one aspect or another.

For a document to be novelty-destroying, it should disclose the existence of the claimed DNA polymerase and provide repeatable teachings for its isolation. This was clearly not the case here. Novelty was also not at stake on the basis that the inevitable outcome of the process disclosed in any of the aforementioned documents would be the full-length polymerase because, in accordance with the case law (see decision T 793/93 of 27 September 1995), a much stricter standard of proof than the balance of probability had to be used in deciding that the inevitable outcome of an express literal disclosure in a particular prior art document was novelty-destroying, which standard of proof had not been met.

For these reasons, the subject-matter of claim 1 and of dependent claims 2 to 4 or claims 27 and 28 (both in part) dependent on/making reference to claim 1 was novel.

- Claim 18

The recombinant DNA polymerase of claim 18 was that produced in E. coli. Document (276a) disclosed that the N-terminal end of the amino acid sequence of this enzyme differed from that of the natural Taq polymerase. Thus, prior sales of the natural Taq polymerase could not be damaging to the novelty of the claimed subject-matter.

Article 56 EPC; inventive step; claim 5

- The relevance of prior sales of the native Taq polymerase

There was no convincing evidence on file that the full-length native Taq polymerase had been sold before the priority date of claim 5 since the one polymerase which had been sold did not carry the same identification number as the one polymerase which had been disclosed as being full-length. As a consequence, prior sales were not of relevance to inventive step.

- Inventive step over the teachings of the prior art

Document (76) represented the closest prior art. The technical problem was to find an enzyme from a thermophilic organism which survived undamaged through the heating part of the PCR cycles.

At the priority date, the person skilled in the art would have turned to document (13) which was the most recent of the publications relating to the enzyme from T. aquaticus, ie would have been convinced that the DNA polymerase would have had a small molecular weight of about 62,000 daltons. This perception would have been reinforced by the results earlier published by the Trela group (see documents (3), (9) and (10)) as well as other results concerning the molecular weight of DNA polymerases from other Thermus species. Thus, the skilled person being at the same time cautious and conservative would have had no incentive to look for a DNA polymerase of a much higher molecular weight, such as the now claimed full-length Taq DNA polymerase. Had the skilled person looked for this enzyme, he/she would have had no reasonable expectation of success to find it. Years later, document (44) taught that it was only by increasing the amount of cells used as starting material for the purification that the full-length enzyme could be detected by staining. As for the technique of *in situ* activity gels, it did exist at the priority date but was only used in relation to purified enzymes as could be derived from documents (112), (218) and (23).

There was no reason for the skilled person to combine the teachings of document (3) or document (9) with that of document (28) since in this latter document the full-length enzyme as isolated in the presence of proteases was of small molecular weight.

The full-length Taq DNA polymerase was inventive. As a logical consequence, also the use for polymerase chain reactions of the inventive enzyme or composition or a

method for the amplification of nucleic acid sequences using the same was inventive.

XIII. The respondents' arguments in writing and during oral proceedings, insofar as they are relevant to the present decision, may be regrouped and summarised as follows:

Admissibility of the 43 documents filed on 29 August 2003; requests for reimbursement of costs

These documents (more than one thousand pages) could have been filed with the statement of grounds of appeal. Filing them at such a late stage in the proceedings was unfair to the parties, especially to those who had not taken part in the previous litigations where most of the documents had originally been filed. It amounted to an abuse of procedure which justified an apportionment of costs.

Main request for all designated Contracting States except AT and ES

Rule 57a EPC

Claims 26 to 32 were not present in the granted claim request and it was not obvious that they were filed in answer to any grounds of opposition under Rule 57a EPC. They should not be allowed.

In particular, new claims 30 to 32 being directed to a stable enzyme composition with specific properties in fact pertaining to a recombinant enzyme (granted

claims 27 to 29) did not correspond to any embodiments of granted claim 30.

Article 123(2) EPC; added matter; claim 1

Claim 3 as filed defined the DNA polymerase by, in particular, its molecular weight (about 86,000 to 90,000 daltons). This molecular weight could only be an absolute molecular weight because the claim did not specify the markers/methods for molecular weight determination. The description left no room for any other interpretation since it did not even hint that the specific molecular weight range experimentally determined for one specific polymerase could be generalised to others as presently claimed in claim 1. In fact, the specification taught that molecular weights could be measured by different methods. Thus, the subject-matter of claim 1 was not supported in the application as filed for all imaginable species.

The present case was not alike that dealt with in decision T 493/94 (see supra) since the invention in this earlier case was **a single protein** with different sialic acid residues. It was then fully justified to accept that the various forms of the protein had the same specific activity, which implied that each of them could be claimed to have that activity, even if only the activity of the mixture had ever been determined. In the present case there was absolutely no ground to consider that the polymerases from different organisms would necessarily have the same properties.

Auxiliary request for all designated Contracting States except AT and ES

Article 123(2) EPC; added matter; claim 1

YT1 was the only strain of Thermus aquaticus referred to in the application as filed. Therefore, there was no support in said application for the subject-matter of claim 1 which encompassed DNA polymerases obtainable from Thermus aquaticus strains other than YT1.

Article 84 EPC; clarity

The use of the expressions "*having DNA polymerase activity*" in claims 16, 18 and 27 (see section X supra) and "*fragment of/thereof*" or "*modification thereof*" in several of the claims rendered the claimed subject-matter unclear.

Article 83 EPC; sufficiency of disclosure in relation to the Taq DNA polymerase

The skilled person could not identify the claimed DNA polymerase on the basis of the teaching in the patent specification because it did not disclose the conditions under which the SDS-PAGE was to be run to obtain the given molecular weight. It was clear from document (244) that the apparent molecular weight measured by SDS-PAGE could vary substantially depending on said conditions. This had even been proven in document (244) in the specific case of the Taq DNA polymerase.

The molecular markers considered as a whole were not appropriate in that five of them had a molecular weight

lower and only one of them had a molecular weight higher, than the molecular weight of the polymerase.

Article 87 EPC; entitlement to priority

For a priority date to be validly allocated to a claimed subject-matter, the relevant priority document had to contain an enabling disclosure of said subject-matter. Only the priority document US 63509 contained a passage equivalent to Example VI of the patent (in which the purification of the Taq polymerase was described). Therefore, the valid priority date for this enzyme (claims 1 to 4) was 17 June 1987. This was also the case insofar as the claims to its uses were concerned (see claim 27 (in part),and claim 28 (in part)).

Article 54 EPC; novelty; claims 1 and 18

- Claim 1

Claim 1 was directed to a natural product, ie to a product which, because it already existed in nature, could not be regarded as new at the date of the invention. Moreover, in view of document (200), the appellant was trying to get a patent for an invention which had been the subject-matter of a previous patent application.

Document (3) disclosed a 62,000 daltons DNA polymerase which could not be a molecule different from the full-length DNA polymerase because essentially the same protocol had been used for its isolation as was taught in the patent in suit. In particular, the enzyme could

not be any of the other two forms of the DNA polymerase which were known at that time (sTaq and Stoffel fragment) because the Stoffel fragment was eliminated by the way the purification process was carried out and sTaq was not formed under the conditions used in document (3). The apparent discrepancy in molecular weight was simply due to the fact that different methods were used to measure the molecular weights. In any case, the skilled person would not have taken the 62,000 daltons molecular weight at face value since document (3) contained a warning as to its possible lack of significance. In document (30) (see page 7322), the identity of the enzyme described in document (3) with the commercial full-length enzyme was not doubted and the observed difference was simply attributed to a difference in tertiary conformation.

Document (40) together with document (230) provided no less than five reproductions of the process described in document (3) which all led to the production of preparations containing full-length DNA polymerase. These reproductions were faithful since, if some steps were added or conditions altered, these steps were either of no consequence or did not concern the purification *per se*. In fact, in the reproduction of the protocol described in document (3) (as reported in document (230), only two deviations could be identified, one being the weight of cells in the crude extract and the other being the height at which the DEAE-Sephadex A-50 column was packed.

The method of *in situ* activity gels used to identify the protein as being full-length Taq polymerase was well known at the priority date.

The same results as those obtained in document (230) were described in documents (43) and (44).

In the same manner, documents (9) and (10) were enabling for the production of the full-length enzyme. This was confirmed by reproducing said processes in documents (40) and (44).

In any case, there were documents on file (eg document (47), Exhibit 3) which showed that even if the experimental conditions for the purification were changed, full-length polymerase was always obtained.

One of the authors of document (13) freely admitted in his declaration (document (37)) that he had originally misinterpreted his own experiment, insofar as he had attributed to the DNA polymerase the molecular weight of 62,000 daltons. This was further confirmed in document (47) (see paragraphs 7 and 8 thereof). The protocol described in document (13) as reproduced in document (44) resulted in a DNA polymerase with the same molecular weight as the one claimed, using *in situ* activity gels.

Each of documents (3), (9), (10) and (13) taken on its own destroyed the novelty of the subject-matter of claim 1.

- Claim 18

The claimed enzyme was obtained by a recombinant process which might have implied that a higher level of purity was achieved than for the enzyme disclosed in

the prior art (see documents (3), (9), (10) and (13)). Yet, the enzyme sold by New England Biolabs (NEB) before the priority date of claim 18 was suitable for PCR and, therefore, was also of a high purity level. The NEB enzyme was novelty-destroying for the subject-matter of claim 18.

Article 56 EPC; inventive step; claim 5

- The relevance of prior sales of the natural Taq polymerase

Lot 1 of the native full-length Taq polymerase was available to the public before the priority date of claim 5. Evidence thereto was to be found in document (47) (see Exhibit 3) which disclosed the purification protocol for the full-length enzyme and its production date (March 1987). In the same manner, document (79), a data sheet, showed that the enzyme was assayed on 20 March 1987. Documents (80) and (81) demonstrated that it was at the disposal of the firm Cetus on that date.

This full-length enzyme was necessarily the same enzyme as the one which was sold to at least six institutions/firms also in March 1987 (see document (78)). The fact that the sold enzyme was not identified as "Lot 1" was of no consequence since, at that point in time, only one batch of the enzyme had ever been produced.

Starting with the available full-length native enzyme, it would have been a matter of routine to clone the

corresponding gene. The subject-matter of claim 5 was not inventive.

Article 56 EPC; inventive step over prior art documents

(Different approaches to inventive step were used by the respondents; they are summarised below.)

– Document (13) was the closest prior art. Upon reading the two first paragraphs of the document, the skilled person would have been prompted to investigate the properties of the DNA polymerase contained in the enzyme preparation described therein, all the more so that there existed at the priority date a great interest in cloning the encoding gene.

If problems were encountered, the skilled person would have turned to document (9) which was cited in document (13) (reference 4). He/she would thus have decided to substitute the chromatography columns and stabiliser used in document (13) by those employed in document (9). He/she would also have added a phosphocellulose column step as the last step since the textbook document (17) described such a step in relation to E. coli DNA polymerase I. By carrying out such a protocol, he/she would have inevitably obtained the full-length Taq DNA polymerase. The changes would have not removed or destroyed the DNA polymerase activity. Identifying the full-length DNA polymerase enzyme could have been achieved with a reasonable expectation of success using *in situ* activity gels. As obtaining the full-length enzyme

was not inventive and the cloning of the gene could be achieved in an obvious manner, the subject-matter of all claims lacked inventive step.

– At the priority date, the person skilled in the art had sufficient incentive to look for enzymes useful in PCR. Document (3) represented the closest prior art and since it mentioned on pages 60 and 61 that the then isolated polymerase could be a fragment of the natural enzyme, he/she would immediately have attempted to obtain the full-length molecule. Document (28) (see page 4823) taught that protease digestion was frequently observed with various DNA polymerases and that protease inhibitors could prevent this proteolysis. It also described useful techniques such as *in situ* activity gels. By combining the teachings of documents (3) and (28), the skilled person would have obtained the full-length enzyme in an obvious manner. There would be no difficulties in detecting the enzyme in the polyacrylamide gel since the technique of *in situ* activity gels had been described (see documents (112) and (218)) and even used to characterize other DNA polymerases (see document (23)). This technique could detect picograms of enzyme. The same reasoning was also valid starting from document (9) or document (10). Furthermore, the possibility of using the DNA polymerase as a starting point in the isolation of the corresponding genes was also contemplated in document (10) (see pages 68 and 69). Therefore, the claimed enzyme was also obvious in view of either of those documents.

– Document (76) was the closest prior art which provided the incentive to obtain a thermostable enzyme. Document (3) warned that the 62,000 daltons Taq polymerase may not be the full-length enzyme. In 1986 for the above mentioned reasons, the skilled person would have been able to purify further the enzyme disclosed in document (3) and to detect it. In any case, the cloning of the corresponding gene could be achieved in an obvious manner starting from the protein fragment described in document (3). The DNA could be cloned in the ëgt11 system described in 1983 (see document (85)). A DNA probe could be derived from the microsequencing of part of the protein fragment (see document (93)). Alternatively, clones expressing the enzymes could be detected immunologically, any protein fragment being suitable as an immunogen (as described in document (110)). Accordingly, the skilled person had a reasonable expectation of success to obtain the native enzyme as well as the Taq polymerase gene. The subject-matter of all claims, thus, lacked inventive step.

XIV. The appellant requested that the decision under appeal be set aside and that the patent be maintained on the basis of either the main request as filed with the statement of grounds of appeal or on the basis of the auxiliary request filed during the oral proceedings.

XV. The respondents requested that the appeal be dismissed. Respondent II additionally requested apportionment of costs.

Reasons for the Decision

Admissibility of the documents filed by the appellant together with its observations of 29 August 2003

1. The appellant filed 43 documents some seven weeks before the oral proceedings, in addition to the at least 163 documents already on file, this filing being within the time limit which the board had set. Said 43 documents amount to more than one thousand pages.

2. In accordance with the case law of the Boards of Appeal (eg decision T 950/99 of 11 November 2002, point 4 of the reasons), although, in principle, an appeal should be essentially based on facts and evidence which were already available to the department of first instance, parties in their effort to make a full statement of the grounds why the revision of the contested decision is requested often rely on additional evidence. Such evidence, especially when filed at the onset of the appeal, is not necessarily defined as being "late-filed". Much depends on its **prima facie relevance**, the board being empowered essentially either (i) to disregard it under Article 114(2) EPC or (ii), having admitted it, to remit the case to the department of first instance under Article 111(1) EPC for further prosecution, or (iii) having admitted it, to decide on the case.

3. This board, keeping in mind that one of its major tasks in *inter partes* proceedings is to safeguard the principle of equal rights to the parties which require that all parties be treated fairly and, in particular, be given the same opportunities to defend their case, considers it appropriate to exercise its discretion under Article 114(2) EPC to accept or refuse the above mentioned documents in the light of the following criteria.

4. A document will be considered of *prima facie* relevance (and, thus, admissible) if it is possible to identify its technical content in a straightforward manner and to assess without difficulties its relevance to the points of law under discussion, ie to evaluate quickly and with reasonable certainty that it has a potential bearing on the decision which is to be taken. The document should, thus, be of a reasonable size, easily readable and written by a technical expert. Furthermore, in accordance with the case law, the nature of the document (newly filed experimental data, technical content...) will also be taken into consideration (see decision T 397/02 of 10 October 2003).

5. Thus, are not accepted in the proceedings:

- statements including declarations, affidavits and transcripts of testimonies made for the benefit of other jurisdictions, following a different set of laws and having a different case law, which are accompanied by very voluminous and often hardly readable annexes, the reading of which is essential to evaluate the relevance of these statements (31 documents);

– statements of non-scientists which cannot be regarded as technical evidence from qualified experts (2 documents);

– thick laboratory notebooks which are almost undecipherable (2 documents);

– new experimental evidence which, in accordance with the EPO practice, may not be filed at such a late stage (2 documents); and

– documents which are of insufficient technical content for helping the board in reaching its decision as they are very old or report informal exchanges between scientists (3 documents).

6. Are accepted in the proceedings **documents (273) and (281)** which are short declarations by technical experts accompanied by a small number of easily readable exhibits and **document (276a)**, a publication in a scientific journal dated 1993 to be taken as an expert's document.

Main request for all designated Contracting States except AT and ES

- Rule 57a EPC in relation with newly filed claims 26 to 32

7. The board accepts the appellant's argument that the product-by-process claim 26 was introduced in the main request under Rule 57a EPC in an attempt to deal with the objection of lack of novelty raised on the basis of

prior art documents disclosing the purification of a DNA polymerase directly from Thermus aquaticus.

8. The subject-matter of granted claim 30 which comprises many independent embodiments (see section I, supra) was redistributed in claims 27 to 32. These claims correspond to granted claim 30 referring to, respectively, (i) granted claim 1 (see claim 27), (ii) granted claims 24 and 25 (see claim 28 part (a)), (iii) granted claim 24 (see claim 28, part (b)), (iv) granted claim 26 (see claim 29) and (v) granted claims 27 to 29 (see claims 30 to 32). This redistribution does not bring any changes in the claimed subject-matter. The introduction of the new claims is accepted as making said subject-matter more easily identifiable as in granted claim 30.

9. The main request is allowable under Rule 57a EPC.

- Article 123(2) EPC; added matter; claim 1

10. The issue at hand is whether or not there is support in the application as filed for thermostable DNA polymerases with a molecular weight as defined in claim 1 (see section IV, supra), other than the one obtainable from Thermus aquaticus.

11. Claim 3 as filed (see section I, supra) relates to DNA polymerases having a molecular weight of 86,000 to 90,000 daltons. In the patent application as filed, it is disclosed that stable polymerases may be obtainable from a number of sources (see page 13, lines 28 to 36) and that the one obtainable from the bacterium Thermus aquaticus has a molecular weight of 86,000 to 90,000

daltons when determined in a SDS-PAGE system using as molecular weight markers phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400)(see page 58, lines 27 to 33).

12. The application as filed is, however, silent about the molecular weight of other thermostable polymerases. In particular, it cannot be said that the passage on page 3, lines 11 to 13: "*In addition to the gene encoding the approximately 86,000 to 90,000 dalton enzyme, gene derivatives encoding DNA polymerase activity are also presented*" amounts to an implicit but unambiguous disclosure of DNA polymerases encoded by genes from any other source than T. aquaticus, since the *"gene"* in question is defined directly above as being "*[t]he gene encoding the enzyme from* [sic] *DNA polymerase from Thermus aquaticus*". In the same manner, the process for the purification of a DNA polymerase detailed from page 14, line 32 to page 16, line 14 and ending with the statement: *"The molecular weight of the dialyzed product may be determined by any technique, for example, by SDS-PAGE using protein molecular weight markers*" does not amount to disclosing a group of DNA polymerases having a molecular weight of 86,000 to 90,000 daltons because the molecular weight is not disclosed, nor does it amount to disclosing a molecular weight experimentally determined as indicated in claim 1, since any technique may be used and, if SDS-PAGE is used, the protein markers are not specified.

13. Consequently, the application as filed does not contain any information which would enable the skilled person

to interpret originally filed claim 3 as an implicit disclosure of the subject-matter of claim 1.

14. By combining various portions of the application as filed including the specific experimental information given in Example I, part III relating to the T. aquaticus DNA polymerase, the appellant was able to come to the conclusion that the description *per se* provided a basis for the subject-matter of claim 1 (see section XII supra). In accordance with the case law (see eg decisions T 157/90 of 12 September 1991 and T 397/89 of 8 March 1991), if the application as filed only describes a specific feature and the feature's general applicability is not evident to the skilled person, then a generalisation cannot be allowed. In the board's judgment and for the reasons given in point 12 (see supra), it is not evident from the teaching of the application as filed that the feature of the molecular weight as specifically mentioned in Example I in relation to the T. aquaticus DNA polymerase can be generalised to all other DNA polymerases. Thus, the board cannot agree with the appellant's conclusion.

15. Finally, the appellant pointed out to decision T 493/94 (see supra) as dealing with a situation comparable to that in the present case, which had been decided in favour of the then patentee insofar as compliance with the requirements of Article 123(2) EPC were concerned. In the board's judgment, this earlier case is clearly different from the present case because the claim then at stake was directed to **one enzyme** defined by the same activity as a mixture of three forms of this very same enzyme. The then reached conclusion that the three forms of the enzymes had the same specific activity

(which was that of the mixture of the three) led the board to accept that the disclosure of any one of the enzymes as having said activity did not constitute added subject-matter although only the specific activity of the mixture had ever been mentioned in the application as filed. This conclusion can obviously not be transferred to the present case since claim 1 is directed to **a group of enzymes** of different sources for which there is no reason to conclude that they should have the same structural properties (such as molecular weight).

16. Thus, the skilled person taking into consideration the whole application as filed, ie not only the description but also the claims and the drawings, can only conclude that it does not disclose either implicitly or explicitly any thermostable polymerases, other than the DNA polymerase obtainable from Thermus aquaticus, having a molecular weight of 86,000 to 90,000 daltons as determined according to the migration of the enzyme in SDS-PAGE, when the marker proteins are phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400).

17. The patent was amended in such a way that it contains subject-matter which extends beyond the content of the application as filed. Therefore, the main request is rejected as not fullfiling the requirements of Article 123(2) EPC.

Auxiliary request for all designated Contracting States except AT and ES

- Article 123(2) EPC; added matter; claim 1

18. The issue at hand is whether or not there is support in the application as filed for thermostable DNA polymerases with the features given in claim 1 (see section X, supra) originating from Thermus aquaticus strains whereas the enzyme was only purified from the Thermus aquaticus strain which is referred to in the patent as YT1 (also designated "YT-1").

19. From the whole paragraph on page 16, lines 12 to 17, as filed, the skilled person is aware that the stable DNA polymerase obtainable from Thermus aquaticus has a molecular weight of 86,000 to 90,000 daltons as determined according to its migration in SDS-PAGE using molecular weight markers.

20. From page 58, lines 27 to 33, as filed, the skilled person is informed that the molecular weight of the stable DNA polymerase extracted from strain YT1 of Thermus aquaticus was estimated to be of 86,000 to 90,000 daltons as determined using as markers phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400).

21. On page 14, lines 1 to 6, as filed, mention is made of several strains of Thermus aquaticus, YT1 being a preferred one amongst them. Thus, YT1 is regarded as a type strain representative of many others. Therefore, the skilled person would have concluded that DNA polymerases could be obtained from various Thermus aquaticus strains, which would have a molecular weight

of about 86,000 to 90,000 daltons as determined according to their migration in SDS-PAGE using the six aforementioned molecular weight markers.

22. The description as filed provides an implicit but unambiguous disclosure of the subject-matter of claim 1 of the auxiliary request. No other objections were raised under Article 123(2) EPC against any claims of this request. In the board's judgment, the subject-matter of claims 1 to 28 finds a basis in the application as filed. Thus, the requirements of Article 123(2) EPC are complied with.

- Article 84 EPC; clarity

23. Objections for lack of clarity were raised for the reason that in each of claims 16, 18 and 27 (see section X, supra), the expression "*enzyme having DNA polymerase activity*" was used whereas the Thermus aquaticus DNA polymerase was meant, with the result that the skilled person had no idea of how broad the scope of the claims was. Further, it was argued that the presence of the expression "*fragment of/thereof*" or, of the expression "*modification thereof*" in, respectively, claim 5, claims 18 and 21 (see section X, supra) and claims dependent thereon or containing a back-reference thereto rendered them unclear.

24. Claim 16 is directed to a method which comprises the culturing of a host cell of claim 14 or claim 15. As claims 14 and 15 are dependent on claim 5 via a succession of dependent claims and claim 5 is explicitly directed to a DNA sequence encoding "***a thermostable Thermus aquaticus DNA polymerase"*** or a

fragment thereof, there can be no doubt that in claim 16 what is meant under the expression "*enzyme having DNA polymerase activity*" is the "*Thermus aquaticus DNA polymerase*". Therefore, the subject-matter of claim 16 is considered to be clear although multi-dependent.

25. The same conclusion also applies to claim 18 because it relates to an enzyme obtainable by a method an essential technical feature of which is the use of a host cell as defined in claim 15.

26. As the thermostable enzyme having DNA polymerase activity referred to in claim 27 is defined as being one of claims 1 to 4, and each of claims 2 to 4 is dependent on claim 1, and claim 1 is directed to a thermostable Thermus aquaticus DNA polymerase, there can be also no doubt that in claim 27 what is meant under the expression "*enzyme having DNA polymerase activity*" is the "*Thermus aquaticus DNA polymerase*". Similarly, the "*recombinant thermostable enzyme of claim 18*" as also recited in claim 27 can be nothing other than a recombinant Thermus aquaticus DNA polymerase (see point 25, supra). Therefore, also the subject-matter of claim 27 has to be regarded as unambiguously defined.

27. The presence of the expression "*fragment of/thereof*" or of the expression "*modification thereof*" in several claims of the auxiliary request is not the result of amendments carried out during the opposition and appeal proceedings. Said terms were already present in corresponding granted claims. Therefore, their use in

the claims of the auxiliary request is not open to discussion for lack of clarity at the appeal stage.

28. The requirements of Article 84 EPC are fulfilled.

- Article 83 EPC; sufficiency of disclosure in relation to the Taq polymerase

29. Respondents II and III contended that the invention was insufficiently disclosed within the meaning of Article 83 EPC because there was no indication in the patent specification as to which SDS-PAGE system was used when the molecular weight of the Taq DNA polymerase was estimated to be of 86,000 to 90,000 daltons.

30. The relevant passage in the patent specification is on page 25, lines 10 to 12 where it is indicated that the molecular weight of the DNA polymerase obtainable from Thermus aquaticus was determined by SDS-PAGE using six particular marker proteins. The conditions under which the electrophoresis was run are, however, not identified.

31. According to document (44) (see paragraph 17 on page 6), there are several parameters, such as the acrylamide and bisacrylamide concentrations or the composition of the various buffers, which can affect the relative electrophoretic mobility of a protein when carrying out a SDS-PAGE determination, and, thus, influence the estimation of its molecular weight.

32. Nevertheless, in document (61) (see paragraphs 11 to 15 on pages 5 to 7) the point is stressed that, by the

filing date of the patent, SDS-PAGE was one of the most common and routine techniques in biochemistry laboratories worldwide, the technique being so standardised that ready-made polyacrylamide gels of different percentage acrylamide were available from manufacturers for analysis of different sized proteins. Standard SDS-PAGE conditions were set out by Laemmli in 1970 and implemented by Weber et al. in 1972 (see Exhibits E and F, respectively, to document (61)). At the filing date, those skilled in the art knew that an accurate molecular weight estimation required a linear standard curve generated by plotting the relative mobility (the distance which the protein has moved into the gel) versus the logarithm of protein's molecular weight, values which are inversely proportional to one another over the range of the molecular weight markers. If one did not know which polyacrylamide gel conditions were appropriate, one could certainly have started with the Laemmli conditions and have varied the percentage acrylamide and the amount of cross-linker bis-acrylamide to obtain polyacrylamide gel conditions under which the specified molecular weight markers generate a linear standard curve.

33. According to document (244), 73% of the scientific papers published in the first two 1986 issues of *"The Journal of Biological Chemistry*", acknowledged as widely accepted as the leading journal in the area of protein biochemistry, described their SDS-PAGE system as a Laemmli system, 29% of those having altered some of the running conditions.

34. For these reasons (see points 32 and 33, supra), the board concludes that the Laemmli SDS-PAGE system was

the predominant one at the filing date, which the skilled person would know how to adapt to suit his/her experimental requirements.

35. In the absence of any experimental evidence from the respondents showing that estimating the molecular weight of the DNA polymerase obtainable from Thermus aquaticus to be of 86,000 to 90,000 daltons when employing the six marker proteins referred to in the patent would have needed unusual SDS-PAGE conditions, the board is of the opinion that at the filing date conditions derivable without undue burden from the basic teaching as set out by Laemmli (see supra) and implemented by Weber et al. (see supra) were appropriate to determine the claimed estimated molecular weight in a repeatable and reliable manner.

36. Finally, also the argument that the set of marker proteins referred to in claim 1 did not enable the skilled person to perform a clear and unambiguous estimation of the molecular weight because those markers did not exactly flank the range of 86,000 to 90,000 daltons, five markers having a molecular weight lower than the range and only one marker having a molecular weight higher, is not tenable. Indeed, what is essential to perform an accurate molecular weight estimation is that a linear standard curve including the expected molecular weight could be generated (see point 32, supra), a requirement which is met by the invention.

37. Therefore, the requirements of Article 83 EPC are complied with.

- Article 87 EPC; entitlement to priority

38. Two priority dates are claimed, namely 22 August 1986 which is the filing date of the priority documents US 899513 and US 899241, and 17 June 1987 which is the filing date of the priority documents US 63647 and US 63509.

39. All parties agree that the inventions relating to DNA sequences, recombinant enzymes, stable enzyme compositions and uses thereof (see claims 5 to 26, claim 27 (in part) and claim 28 (in part)) are entitled to the latter priority date (17 June 1987). The board also shares this opinion. The priority date of the claims directed to the Thermus aquaticus enzyme *per se* and to its uses (see claims 1 to 4, claim 27 (in part) and claim 28 (in part)) remains to be determined.

40. According to decision G 2/98 (OJ EPO 2001, 413) the requirement for claiming priority of "*the same invention*", referred to in Article 87(1) EPC, means that priority of a previous application in respect of a claim in a European patent application in accordance with Article 88 EPC is to be acknowledged only if the skilled person can derive the subject-matter of the claim directly and unambiguously, using common general knowledge, from the previous application as a whole.

41. The board notices that the passages in the priority document US 899241 relating to the purification of the Thermus aquaticus enzyme, to its use for polymerase chain reaction and to a method for the amplification of nucleic acid sequences comprising the same use (see pages 29 to 41; Examples I to III) are the same as the

corresponding passages in the patent in suit (see pages 24 to 29; Examples I to III). When discussing sufficiency of disclosure, it was never argued that the purification steps of the claimed Thermus aquaticus enzyme (see claims 1 to 4) was insufficiently disclosed but only that it could not be identified with certainty (see point 29, supra). It was not challenged that its uses (see claims 27 and 28, in part) could be carried out on the basis of the information given in the patent specification. The priority document US 899241 containing the same information as the patent in suit in this respect, it must follow that it provides an enabling disclosure of the subject-matter of claims 1 to 4, claim 27 (in part) and claim 28 (in part).

42. The argument that claim 1 could not be entitled to the earlier priority date of 22 August 1986 because the claim encompassed a recombinant Thermus aquaticus DNA polymerase is not accepted, the reason being that claim 1 is directed to a product, whatever the method used to produce it and that, as just mentioned, one such method was enabled at the priority date.

43. For these reasons, it is decided that claims 1 to 4, 27 and 28 relating to the Thermus aquaticus enzyme are entitled to the priority date of 22 August 1986.

- Article 54 EPC; novelty

- claim 1

44. The four documents (3), (9), (10) and (13) belonging to the state of the art according to Article 54(2) EPC

were argued to be novelty-destroying for the subject-matter of claim 1.

45. **Document (3)** (Edgar thesis) describes the purification of a DNA polymerase from a crude extract of a bacterial material without precise identification (see page 14, first paragraph which recites "*The bacterium used in these experiments was Thermus aquatius* [sic], *an extreme thermophile, kindly supplied by Dr. Paul Ray, University of Kentucky.*"). The protocol includes three consecutive chromatography steps on a DEAE-Sephadex A-50 column, a phosphocellulose column and, then, a DNA-sepharose column (see page 36). When the active fraction eluted from this last column (Fraction V) is run **on a sucrose gradient**, the enzyme appears as a single peak with a sedimentation coefficient of 5.9s, corresponding to **an estimated molecular weight of 72,000 daltons** (see page 52).

46. The purification protocol described in **document (9)** (Chien paper) which starts with a crude extract of cells of the Thermus aquaticus **YT-1** strain also includes three consecutive chromatography steps on, respectively, a DEAE Sephadex A-50 column, a phosphocellulose column and a DNA-cellulose column. The molecular weight of the DNA polymerase contained in the resulting active eluted fraction (Fraction IV) is estimated **by sucrose gradient centrifugation** to be **68,000 daltons** and **by gel filtration** to be approximately **63,000 daltons** (see pages 1554 and 1555).

47. **Document (10) (**Chien Thesis) describes a purification procedure which also starts from a crude extract of **YT1** cells. To three chromatography steps involving the same

material as the procedure of document (9) (giving a Fraction IV), two chromatography steps are added, namely, a chromatography on hydroxyapatite (giving a Fraction V) followed by a chromatography on a phosphocellulose column. The molecular weight of the DNA polymerase contained in Fraction IV is estimated to be of about **68,000 daltons by sucrose gradient centrifugation** (see pages 32 and 58). The molecular weight of the enzyme in Fraction V is shown to be of about **63,000 daltons as measured by gel filtration** using a Sephadex G-100 column (see pages 34 and 60).

48. **Document (13)** (Kaledin et al.) describes a purification procedure starting with a crude extract of **YT1** cells involving an ammonium sulfate fractionation, four consecutive chromatography steps on, respectively, a DEAE-cellulose column, an hydroxyapatite column, a further DEAE-cellulose column and a single-strand DNA-cellulose column. By performing a **SDS-PAGE** on the active fraction eluted from this last column (Fraction VI), it was determined that the molecular weight of the DNA polymerase was of **about 62,000 daltons** (see page 500).

49. There is no doubt that none of these documents provides an explicit disclosure of the DNA polymerase of claim 1. Thus, assessing novelty amounts to answering whether or not the claimed subject-matter can be inferred directly and unambiguously (albeit implicitly) from the disclosure in any one of the four documents (see eg decision T 465/92, OJ EPO 1996, 32). In accordance with the case law (see decision T 666/89, OJ EPO 1993, 495), if carrying out a process specifically or literally described in a prior art document inevitably results in

a product which is not described, then this amounts to a disclosure which deprives of novelty a claim covering said product. In order that the "inevitable link" between the process and the product be established, it must, of course, be possible to detect the product in a clear and unambiguous manner.

50. When drawing a conclusion from the analysis made above (see points 45 to 48, supra), the skilled person would make a clear distinction between document (13) and the other three documents ((3), (9) and (10)).

51. Document (13) describes a preparation (Fraction VI) containing a DNA polymerase, the molecular weight of which is determined to be about 62,000 daltons, using SDS-PAGE like in the patent in suit. There can be no doubt in view of this significantly smaller molecular weight that the DNA polymerase does not correspond to an enzyme falling within the definition of the DNA polymerase according to claim 1. The fact that one of the authors of document (13) declared some eighteen years later (see document (37)) that he had erroneously assumed that the 62,000 dalton protein was the Taq DNA polymerase cannot change the teachings of document (13) on its effective date. Moreover, account should also be taken of the confirmation made by another co-author of document (13) (see document (281)) that the preparation of document (13) as obtained by Kaledin et al. contained a Thermus aquaticus DNA polymerase enzyme having a molecular weight estimated by SDS-PAGE to be 62,000 daltons.

52. Therefore, the board concludes that document (13) does not disclose a process which inevitably results in the DNA polymerase of claim 1 and, thus, that it is not novelty-destroying for the subject-matter of said claim.

53. A number of experiments which purport faithfully to reproduce the purification protocols described in documents (3), (9) and (10) (see points 45 to 47, supra) were filed in order to show that said protocols inevitably resulted in the DNA polymerase of claim 1, ie in an enzyme having a molecular weight of 86,000 to 90,000 daltons when measured on SDS-PAGE.

54. According to decision T 793/93 (see supra), "*in deciding what is or is not the inevitable outcome of an express literal disclosure in a particular prior art document, a standard of proof much stricter than the balance of probability, to wit beyond all reasonable doubt needs to be applied. It follows that if any reasonable doubt exists as to what might or might not be the result of carrying out the literal disclosure and instructions of a prior art document, in other words if there remains a "grey area" then the case on anticipation based on such a document must fail.*" In the board's judgment, a faithful reproduction of an experiment reported in any of documents (3), (9) and (10) can only be one which reproduces as accurately as possible the very same experimental conditions the authors were using, starting from the very same material. The only deviations which may be acceptable should be those resulting from the replacement of a material which no longer exists, provided that those deviations can be proven not to have any influence at all on the outcome of the reproduced experiment.

Deviations made only for experimental convenience are not acceptable.

55. **Reproductions of the purification protocol described in document (3)** are reported in documents (43) and (44) as well as in document (40) (together with document (230)).

56. In document (43), it is mentioned that the Edgar's experiment of document (3) was reproduced without any difficulties and that a DNA polymerase with a molecular weight of 86,000 to 90,0000 daltons had been obtained. This one page document, however, fails to show any experimental protocols and data. It is, thus, considered as a disclosure insufficient to show that the polymerase would be the inevitable outcome of the process described in document (3).

57. In document (44) (see point 38 thereof), the research worker repeating the experiment mentions that: "*...I had the purification repeated at a larger scale to allow more extensive characterization of the enzyme. A number of parameters such as centrifugation rotors utilized, column size and dimensions, column flow rates, etc., were altered as would be expected in a scaled up procedure.*". This repeat is obviously not suited to show that a DNA polymerase such as claimed in claim 1 was the enzyme present in Edgar's Fraction V (see document (3)).

58. Document (40) together with document (230) also reports a repeat of Edgar's protocol described in document (3) carried out with the strain YT1 as a substitute for the starting biological material used by Edgar, which is, as mentioned in point 45 (see supra), solely defined in

document (3) as being "*Thermus aquatius, an extreme thermophile*". The board has doubts that this deviation from Edgar's experimental protocol can be allowed. Indeed, in the absence of any information on the genetic identity of Edgar's strain, one cannot be sure that it would be a T. aquaticus strain which YT1 is the prototype of, ie a strain equivalent to YT1 such as those which are mentioned in the application as filed on page 14 (see also point 21, supra).

59. The findings in documents (40) and (230) will nonetheless be discussed assuming that Edgar's strain was a strain which would be "represented" by YT1.

60. The purification protocol according to Edgar as repeated in document (230) leads, at least in some attempts, to a fraction corresponding to Fraction V. The content of this fraction is characterized by a method named *in situ* activity gel which entails that the enzyme is detected **by its activity** *in situ* in the SDS-polyacrylamide gel once renaturation has been carried out: a protein having DNA polymerase activity and exhibiting a molecular weight comprised within 86,000 to 90,000 daltons is identified.

61. This method, however, is not the only method available to the skilled person to determine the molecular weight of the active moiety in the fraction: one other well-tried method which was already much in use as long ago as the date of publication of document (3) consists in submitting the active fraction to gel electrophoresis, detecting the proteins present **by staining** and determining their molecular weight in relation to a set of markers (see the reference to the Laemmli system,

point 32, supra). In document (44) (see paragraph 38 thereof), the respondents' technical expert explains in relation to this method: *"The purification of Taq DNA polymerase that was reported in Edgar's thesis started with a very small quantity of cells. This scale could not yield enough polymerase to visualize on a Coomassie (a type of non-specific protein stain) stained SDS-PAGE gel.".* By this test, it would, thus, be impossible to show that the Edgar's protocol inevitably resulted in an enzyme having a molecular weight according to claim 1. Otherwise stated, two different methods making use of SDS-PAGE may give two different answers with regard to the characterisation of the enzyme which is the end product of the repeats of the Edgar's protocol.

62. **Reproductions of the purification protocols described in document (9) (Chien paper) and (10) (Chien thesis)** are also found in documents (40) and (44). In document (40), (see points 9 and 10 thereof), the author states that: "*I also purified Taq DNA polymerase according to the protocol set forth in the Chien thesis (this protocol employs essentially the same chromatographic protocol set forth in the Chien paper). I followed the procedures of the Chien thesis exactly with the following exceptions.* [Here follows a description of the parameters which were changed]*... I followed the purification protocol of the Chien thesis through the third chromatographic column (DNA-cellulose). The Chien thesis describes additional chromatography steps....However, most of the enzymatic characterization was performed on the enzyme following the first DNA-cellulose column (Chien paper, p.1552). The Chien paper only describes these three chromatographic steps in detail. Therefore I stopped*

the purification procedure at this point and characterized the enzyme." [passage in normal script added by the board]. From this statement, it can be concluded, firstly, that no enzyme such as the one obtained in document (10) was characterized, if only because the purification was not carried out to the end and, secondly, that one cannot be sure that the enzyme which was characterized corresponded to that obtained in document (9) because the early steps in the purification were altered.

63. The same is true for the reproduction of the process described in documents (9) and (10), in document (44) since it is mentioned in paragraph 41 thereof (as in paragraph 38 thereof in relation to the repeats of Edgar's experiments): "*I had the purification repeated at a larger scale to allow more extensive characterization of the enzyme. A number of parameters such as centrifugation rotors utilized, column size and dimensions, column flow rates, etc., were altered as would be expected in a scaled up procedure."*.

64. Furthermore, and quite irrespective of the fact that the purification procedures were repeated in a faithful manner or not, it remains that the end product of these procedures was characterized by *in situ* activity gels. The reasoning developed in points 60 and 61 (see supra) in relation to Edgar's Fraction V therefore equally applies.

65. From these findings (see points 49, 51, 52 and 55 to 64, supra), it is concluded that:

– none of the documents of the prior art provides an *expressis verbis* disclosure of the enzyme of claim 1;

– document (13) does not disclose the enzyme of claim 1;

– when repeating the purification protocols described in documents (3), (9) and (10), deviations were introduced which imply that these repeats cannot be considered as absolutely identical repeats of the purification protocols in documents (3), (9) and (10) (see points 57, 58, 62, 63, supra), and furthermore,

– the results one may expect when detecting the outcome of the purification protocols as carried in documents (3), (9) and (10), by SDS-PAGE electrophoresis will depend on the detection method used (see points 60 and 61, supra).

66. For these reasons, the evidence provided to show that a DNA polymerase according to claim 1 (ie having a molecular weight of 86,000 to 90,000 as determined according to its migration in SDS-PAGE, when the marker proteins are phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500) and lysozyme (14,400)) would be the inevitable outcome of the purification methods of the prior art and could be straightforwardly identified as such does not meet the required standards (see point 49, supra) and thus, no conclusion of lack of novelty may be reached on this basis in relation to claim 1.

- Claim 1 (further arguments)

67. The argument that the appellant is seeking protection for a natural product which as such previously existed and, thus, could not be regarded as new at the date of the invention is not found convincing. Indeed, from Rule 23c(a) EPC, which specifically states that biological material which is isolated from its natural environment or produced by means of a technical process, **even if it previously occurred in nature**, shall be patentable, it may be inferred that, as a matter of fact, the pre-existence of a product in nature is not as such a reason to deprive it from novelty.

68. The further argument was presented that the claimed enzyme is not new over the teachings of document (200). The patent application corresponding to this document is part of the state of the art under Article 54(3) EPC for all designated Contracting States except ES (the earlier priority date of the patent at issue, ie 22 August 1986, counting as the date of filing). It basically describes the technology now known as the polymerase chain reaction (PCR). The polymerase useful for carrying out this method is "*selected from E. coli DNA polymerase I, T4 DNA polymerase, a heat stable enzyme or reverse transcriptase*" (see claim 5). As far as a heat stable enzyme is concerned, reference is made in the description (see page 7) of the enzyme described in document (13), which enzyme was shown in point 51 (see supra) not to be novelty-destroying for the subject-matter of claim 1. Thus, the subject-matter of claim 1 is novel over the disclosure of document (200).

- Claim 18

69. Claim 18 enjoys the latter priority date (17 June 1987), it was objected to for lack of novelty over prior sales of the native Taq polymerase which, according to the respondents, took place between the first and second priority dates. The claimed recombinant enzyme is produced in E. coli (see section X, supra). Document (276a) (see page 281; to be taken as an expert's document) teaches that: "*Based on amino acid analysis (data not shown), recombinant Taq Pol I (in contrast to native Taq DNA Polymerase) was not blocked at the amino terminus and retained the initiating methionine residue, as would be predicted from the properties of E.coli methionine amino peptidase.*" In the context of this article, the term "recombinant Taq Pol I" means Taq polymerase produced in E. coli. From this information, and even if prior sales of native Taq polymerase had been satisfactorily demonstrated (see points 74 and 75 infra), it can be concluded that said sales would not affect the novelty of the recombinant enzyme of claim 18.

- Remaining claims

70. Claims 2 to 4, claim 27 and claim 28 (insofar as they enjoy the earlier priority date) depend on/contain a back-reference to claim 1. Their subject-matter is also novel. Claim 5 relating to a DNA sequence encoding the polymerase according to any one of claims 1 to 4, or a fragment thereof, was never objected to for lack of novelty. The subject-matter of claims 6 to 17 which are dependent on claim 5 or contain a back-reference to that claim is also novel. The subject-matter of claims

relating to stable enzyme compositions and their uses is novel if only because said compositions contain a buffer comprising one or more non-ionic polymeric detergents (claims 19 to 26, claim 27 (in part) and claim 28 (in part)).

71. The novelty of the subject-matter of the auxiliary request is acknowledged.

- Article 56 EPC; inventive step over prior sales

72. Lack of inventive step of the claimed subject-matter relating to the cloned Taq gene, its expression product and their uses was argued on the basis that prior sales of the full-length native Taq polymerase had occurred before the priority date of said subject-matter, ie before 17 June 1987. In the respondents' opinion, the thus available native Taq polymerase could have been used in an obvious manner as the starting point for a straightforward cloning of the Taq gene. Such an argument, of course, presupposes that prior sales have been satisfactorily documented.

73. In accordance with the case law, the more serious the issue, the more convincing must the evidence be to support it. If a decision on such an issue might result in refusal or revocation of a European patent, for example in a case concerning alleged prior publication or prior use, the available evidence in relation to that issue has to be very critically and strictly examined (see decision T 750/94, OJ EPO 1998, 32). It is furthermore stated in decision T 848/94 of 3 June 1997 that to prove that the subject-matter of a patent in suit has been made available to the public within

the meaning of Article 54(1)(2) EPC by way of prior use, it is necessary to establish:

(a) the date on which the prior use occurred ("when" issue),

(b) exactly what was in prior use ("what" issue), and

(c) the circumstances surrounding the prior use (issue of confidentiality).

Here, the concern is not that of a prior use but of prior sales. However, prior sales are considered as a form of prior use (see decision T 670/00 of 10 December 2002). Thus the same criteria apply.

74. From six invoices filed as part of document (78), it can be inferred that, in March 1987, the firm New England Biolabs (also referred to thereafter as NEB) sold two different products being marked "*Taq DNA Polymerase-500 U*" with catalog number "*252L*" and "*Taq DNA Polymerase-100 U*" with catalog number "*252S*". There is no evidence on file from the institutions/firms which received the enzyme that it was the full-length Taq polymerase.

75. In fact, the evidence provided in support of a full-length Taq polymerase being available to the public prior to the relevant priority date relates to a Taq DNA polymerase called "Lot 1". This evidence originates from two sources:

– from NEB itself: document (79) is a data sheet describing experimental conditions in which to use

the Taq polymerase "Lot 1"; document (47) discloses that "Lot 1" started being sold in March 1987 and has a molecular weight of 90,000 to 98,000 daltons.

– from the firm Cetus: for example, document (80) dated 20 March 1987 describes the use of NEB Taq "Lot 1".

However, the fact that Cetus had at its disposal the full-length Taq polymerase "Lot 1" as early as March 1987 was not *per se* argued to be an evidence that the enzyme was available to the public at that date, which evidence may have been quite a convincing proof thereof. It was simply argued that the existence of the full-length natural Taq polymerase as demonstrated by the combination of documents (47) and (80) necessarily implied that the Taq polymerase sold to the above mentioned firms in March 1987 must also have been the full-length Taq polymerase. In the board's judgment, this last argument clearly points out to a missing factual link between the date of the sales as evidenced by the invoices and the date when full-length Taq polymerase became available to the public.

76. For the reasons given in points 74 and 75 (see supra), it is concluded that the condition (b) mentioned in point 73 (see supra) is not fulfilled. Consequently, prior sales of full-length Taq polymerase have not been documented to the standard required in accordance with the EPO practice for it to be taken as prior art in an evaluation of inventive step.

- Article 56 EPC; inventive step over the prior art

- Claim 1

77. Five documents ((76), (3), (9), (10) and (13)) were mentioned by the respondents as possible closest prior art to the subject-matter of claim 1. The contents of documents (3), (9), (10) and (13) have been described in detail in points 45 to 48 (see supra). Document (76) describes the polymerase chain reaction (PCR) technique for the amplification of a desired DNA sequence. It is mentioned on page 159 thereof, right-hand column: "*Because of the repeated cycles of heating and cooling necessitated the repeated addition of polymerase, Mullis and his colleagues decided to try using an enzyme from a thermophilic organism, which survives undamaged through the heating part of the cycle.*".

78. In accordance with the case law (see eg decision T 606/89 of 18 September 1990), the closest prior art for the purpose of objectively assessing inventive step is generally that which corresponds to a similar use requiring the minimum of structural and functional modifications. In the present case, document (76) is the only one which discloses a method for amplifying nucleic acids and also suggests the use of an enzyme from a thermophilic organism for that purpose. Therefore, it is considered to be the closest prior art.

79. Starting from document (76), the problem to be solved may be defined as the provision of an enzyme suitable in the PCR process, ie which withstands the very high temperature required for the denaturing steps in said

process and, thereby, survives undamaged through the heating part of the cycle.

80. The solution thereto is the enzyme of claim 1 defined by its origin, its molecular weight and its thermostability.

81. In light of document (76), the skilled person wanting to solve the above mentioned problem would obviously turn to the prior art relating to DNA polymerases from thermophilic organisms in the hope that one of them might fulfil the conditions of thermostability. At the priority date, two thermophilic organisms at least had already been used as sources of such enzymes: Sulfolobus acidocaldarius (see document (23)) and Thermus aquaticus (see documents (13), (3), (9) and (10)). Taking into account the fact that documents (3), (9) and (10) were published some 14 and 10 years before the priority date whereas six years separated the publication of document (13) and said date, it is to be expected that the skilled person, interested in using the last organism would primarily focus on document (13). In any case, none of the documents disclose any information on the stability of the enzymes they describe, at the relevant temperature.

82. It is mentioned in document (13), page 498 that the enzyme "*exhibits polymerase activity in a wide range of temperatures - from 45 to **90**° (22 and 28% of the activity at the optimum respectively).*"(emphasis added by the board). Thus, and although it is not clear from the document which fraction in the purification protocol gave this result, the skilled person may nonetheless have been prompted to investigate the

enzyme further. As in document (13), the DNA polymerase activity is attributed to a protein having a molecular weight of 62,000 daltons, the skilled person would test the polymerase activity and thermostability of said protein. What result would then be obtained is unclear in the light of the post-published evidence: either no polymerase activity would be observed because this is the wrong protein (see document (37)) or the protein would indeed be shown to have polymerase activity (see document (281)). If, in addition, the 62,000 dalton protein could be shown to be thermostable, then it would remain that it is a different protein from that claimed in claim 1.

83. Thus, it is concluded on the basis of document (13) either that the isolation of the 86,000 to 90,000 dalton polymerase is surprising or that the skilled person wanting to isolate a thermostable DNA polymerase would be confronted to a situation where he/she would have to exercise inventive skill to find out the reasons for his/her negative results and to find ways, if any, "to correct" it.

84. In this context, it was argued that it would have been obvious to detect the high molecular weight polymerase using *in situ* activity SDS-polyacrylamide gels. The board cannot follow this argument because, firstly, the observed failure would not necessarily have been perceived as being due to the detection method and, secondly, several detection methods were available.

85. The combinations of the teachings of documents (13), (9) and (17), or of documents (3) and (28) were also argued to be damaging to the inventive step of claim 1. For

the above explained reasons (see point 78, supra), neither document (13) nor document (3) is the closest prior art. And besides, document (13) does not in any way suggest that either of documents (9) and (17) contains information which it might be useful to combine with its own teachings in an attempt to achieve a better purification of the enzyme. In fact, document (13) does not mention the necessity for a further purification and, if it refers to document (3), it is only in the very general context of reviewing what was done before 1980 in the field of DNA polymerases from thermophilic organisms. The second combination of documents is unrealistic, seeing on the one hand that document (3) does not contain any information in addition to that which is contained in document (13) and was published eight years earlier and that, on the other hand, document (28) does not even concern Thermus aquaticus but Methanobacterium thermoautotrophicum, an unrelated thermophilic bacterium.

86. For these reasons, inventive step is acknowledged to the subject-matter of claim 1, dependent claims 2 to 4 as well as claims 27 and 28, both in part. The subject-matter of claim 19 enjoying the latter priority date is also inventive as no disclosure took place between the first and second priority date which would lead to the conclusion reached in relation to the subject-matter of claim 1 being reconsidered. The same conclusion applies *de facto* to the subject-matter of claims 24 to 28, insofar as they are dependent on claim 19 or contain a back-reference thereto.

- Claim 5

87. The Thermus aquaticus full-length native DNA polymerase was unknown at the priority date of 17 June 1987. The skilled person had therefore no incentive to isolate the DNA encoding it. Had he/she nonetheless wanted to obtain such a DNA on the sole basis that T. aquaticus must of necessity produce a DNA polymerase, it remains that, in order to clone said DNA, the protein needs to be highly purified: DNA probes can only be derived from the protein sequence and antibodies useful for screening can only be raised against a purified enzyme. As the findings in points 82 to 84 (see supra) lead to the conclusion that obtaining the purified enzyme requires inventive step, it follows that the encoding DNA is not obvious.

88. The argument that the DNA encoding the full-length polymerase would have been obtained in an obvious manner starting from the 72,000 dalton protein described in document (3), since all techniques were available at the priority date which were necessary for the cloning and characterisation of said DNA, cannot be followed. Indeed, in accordance with the case law (see decision T 60/89, OJ EPO 1992, 268), the question is not whether the skilled person **could** have carried out the invention but whether he/she **would** have done so with a reasonable expectation of success. In the board's judgment this last point must be answered by the negative since in document (3) (see page 67), the enzyme is said to have been partially purified.

89. For these reasons, inventive step is acknowledged to the subject-matter of claim 5 and to claims 6 to 17 which are either dependent thereon or contain a back-reference thereto.

- Remaining claims

90. The subject-matter of claim 18, claims 20 to 23 and claims 24 to 28 (these latter claims in part), relates to the Taq DNA polymerase in various forms and to its uses. It cannot be put into practice unless the polypeptide according to claim 1 or the DNA according to claim 5 is available. Accordingly, it is also inventive.

91. The auxiliary request as a whole meets the requirements of Article 56 EPC.

Corresponding claims for the Contracting States AT and ES

92. The same reasoning as developed in points 18 to 91 (see supra) also apply to the corresponding claim request for AT and ES, leading to the same conclusion of patentability.

Description

93. As the amended description filed at the oral proceedings results in an appropriate adaptation of the description of the granted patent to the auxiliary request, which is necessary for a correct determination of the extent of protection as foreseen in Article 69 EPC, the board regards said amended description as acceptable. The respondents did not object to it.

Request for apportionment of costs

94. Respondent II requested apportionment of costs to compensate the costs incurred by the reviewing of the 43 last-filed appellant's documents.

95. In principle, each party to opposition proceedings meets its own costs. However, under Article 104(1) EPC, the board of appeal exercising its discretion may order **for reasons of equity** a different apportionment of costs. According to the case law, apportionment of costs is justified if the conduct of one party is not in keeping with the care required, that is if costs arise from culpable actions of an irresponsible or even malicious nature (see decision T 432/92 of 28 January 1994, point 8 of the reasons).

96. The 43 last-filed appellant's documents admittedly represented a quite important mass of papers. They were filed with a letter dated 29 August 2003 in reply to the board's communication dated 10 April 2003, ie some three weeks before the time limit fixed by the board in that communication to file further evidence if necessary.

97. In the board's judgement, this is not an action which may be defined as a culpable action of an irresponsible or even malicious nature from the part of the appellant which would justify the requested apportionment of costs for reasons of equity. The request is, thus, refused.

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:

   – claims 1 to 28 of the auxiliary request filed at oral proceedings for the designated states BE, CH, LI, DE, FR, GB, GR, IT, LU, NL and SE,

   – claims 1 to 51 of the auxiliary request filed at oral proceedings for the designated states AT and ES,

   – amended description filed at oral proceedings, and

   – drawings as originally filed.

3. The request for apportionment of costs is refused.

The Registrar: The Chairwoman:

A. Wolinski F. Davison-Brunel