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Datasheet for the decision of 15 November 2012

Case Number: T 1705/11 - 3.3.08

Application Number: 96119077.4

Publication Number: 776970

IPC: C12N 9/12, C12N 9/96, C12N 15/00, C12P 19/34, C12Q 1/68

Language of the proceedings: EN

Title of invention:
Stable enzyme composition comprising a thermostable nucleic acid polymerase enzyme

Patentee:
F. Hoffmann-La Roche AG

Opponent:
NEW ENGLAND BIOLABS, INC.

Headword:
Stable thermostable nucleic acid polymerase composition/HOFFMANN-ROCHE

Relevant legal provisions:
EPC Art. 54, 56
RPBA Art. 12(4), 13(1)

Keyword:
"Admissibility of new documents and evidence into the appeal (no)"
"Claims as maintained by the first instance - novelty and inventive step (yes)"

Decisions cited:
T 1080/01

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1705/11 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 15 November 2012

Respondent: (Patent Proprietor)
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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted on 21 June 2011 concerning maintenance of European patent No. 776970 in amended form.

Composition of the Board:

Chairman: M. Wieser
Members: P. Julià
D. S. Rogers

Summary of Facts and Submissions

I. European patent number 0 776 970 is based on European patent application 96 119 077.4, a divisional application of the earlier European patent application 87 307 433.0 published as EP 0 258 017. The patent was granted with five claims and an opposition was filed on the grounds of Articles 100(a) and (c) EPC. Claim 1 as granted read as follows:

"1. A stable enzyme composition comprising a thermostable nucleic acid polymerase enzyme in a buffer comprising one or more non-ionic polymeric detergents."

II. In its interlocutory decision of 21 June 2011, the opposition division decided to maintain the patent on the basis of an Auxiliary Request filed at the oral proceedings of 10 March 2011. The Main Request (claims as granted) was considered to lack novelty over document N1. Claim 1 of the Auxiliary Request allowed by the opposition division read as granted claim 1 with the following purpose-related feature at the end of the claim:

"... for use in a process for amplifying one or more specific nucleic acid sequences present in a nucleic acid or mixture thereof using primers."

Claims 2 to 4 were preferred embodiments of claim 1 defining the concentration of the detergents (claim 2), the specific detergent (claim 3) and the buffer (claim 4). Claim 5 was directed to a method of producing a stable enzyme composition of any of claims 1 to 4.

III. Both, patentee and opponent, filed notices of appeal and their grounds of appeal. The patentee subsequently withdrew its appeal, therefore in this decision the patentee will be referred to as the respondent and the opponent as the appellant. The grounds of appeal contained new documents N22 to N25 (respondent) and N26 to N34 (appellant). The respondent requested that the decision under appeal be set aside and, as a Main Request, that the patent be maintained as granted. Two Auxiliary Request were filed, the first being a request that was not admitted into the opposition proceedings and the second that on which the patent was maintained. The respondent requested that the appeal fee be reimbursed because of a substantial procedural violation and that the board accelerated the processing of the appeal due to a pending infringement suit in Germany. The appellant requested that the decision under appeal be set aside and the patent be revoked.

IV. On 21 March 2012, both parties replied to the other party's grounds of appeal. The respondent filed a new Main Request, Auxiliary Requests I to XVII and new documents N35 to N38.

V. On 30 April 2012, the parties were summoned to oral proceedings and, in a communication annexed thereto, they were informed of the board's preliminary, non-binding opinion on the substantive issues of the case.

VI. On 18 September 2012, the appellant replied to the board's communication and filed new documents N27.2 and

N43 to N48. A further document (N64) was filed on 12 November 2012.

VII. On 12 October 2012, the respondent replied to the board's communication and filed new documents N49 to N63, a new Main Request, which was the request on which the patent was maintained by the opposition division, and Auxiliary Request I to IV. The request for reimbursement of the appeal fee was withdrawn.

VIII. Oral proceedings took place on 15 November 2012. At the end of these proceedings, the patentee withdrew its appeal.

IX. The following documents are cited in this decision:

N1: C. Rüttimann et al., Eur. J. Biochem., 1985, Vol. 149, pages 41 to 46;

N2: MBR Product Information Sheet "DNA Polymerase (*Thermus aquaticus*)";

N4: C.H. Suelter, "A Practical Guide to Enzymology", John Wiley & Sons, Inc., 1985, Chapter 1, pages 1 to 26;

N5: C.H. Suelter, "A Practical Guide to Enzymology", John Wiley & Sons, Inc., 1985, pages 17, 18, 59, 60 and 126;

N27: Declaration of Andrew F. Gardner, signed on 28 October 2011;

N27.2: Figure 2 of document N27, filed on 18 September 2012;

N28: Declaration of Peter J. Smyczek, signed on 20 October 2011;

N31: Extracts from molecular biology catalogues;

N33: EP-A2-0 200 362 (publication date: 10 December 1986);

N39: Master of Science (Thesis) of David Bruce Edgar, University of Cincinnati, A.B. Kenyon College, 1972.

X. The arguments of the opponent, the sole appellant in these proceedings, may be summarized as follows:

Article 100(a) EPC; Article 54 EPC
Admissibility of documents N27 and N27.2 into the appeal proceedings

The filing of an Auxiliary Request with a purpose-related feature on 10 February 2011 was a fundamental change in the factual framework of the case. The granted claims only required the presence of a thermostable polymerase and a non-ionic polymeric detergent. This polymerase was not required to be suitable for amplification, let alone for PCR amplification, and none of the granted dependent claims comprised such a purpose-related feature that was taken from the description of the patent-in-suit. There was no reason for the opponent to foresee the filing of such a claim. Whereas document N1 clearly anticipated

the subject-matter of the granted claims, as shown by the decision of the opposition division on these claims, experimental evidence was necessary in order to show that document N1 also anticipated the subject-matter of the Auxiliary Request filed on 10 February 2011. However, this Auxiliary Request was filed only one month before the oral proceedings at first instance. Thus, lack of time had prevented the opponent from producing the evidence shown in documents N27 and N27.2 before or at these oral proceedings. This evidence had been filed as soon as possible, i.e. with the Grounds of appeal.

The purpose-related feature of claim 1 referred to a mere amplification and not to a PCR amplification for commercial purposes. This amplification did not necessarily have to result in sharp, distinct PCR bands. The patent-in-suit referred to an amplification process yielding a mixture of nucleic acids, resulting from the original template nucleic acid, the expected target amplified products, and various background non-target products. This was also shown in Figure 2 of documents N27 and N27.2, wherein the smear band corresponded to such a mixture, containing the expected amplified product (150 bp) and various background non-target products. This was also in line with the results described in Example IX of the patent-in-suit in which the resulting amplification was described as exhibiting a smear of DNA. The amplification referred to in claim 1 did not exclude this type of amplification nor the presence of other reactions, such as DNA synthesis.

Novelty in the light of documents N1 and N2

Document N1 disclosed the purification and characterization of the DNA polymerases from *Thermus thermophilus* HB-8. At least, the initial steps of the purification method contained a non-ionic polymeric detergent (Brij-58). Documents N27 and N27.2 showed that the dialyzed ammonium sulphate fraction, obtained after cell-lysis and streptomycin sulphate treatment, was suitable for PCR amplification of a specific nucleic acid sequence (M13 template).

Document N2 was a product data sheet from Molecular Biology Resources, Inc. (MBR), with facsimile date June 10, 1987, offering the thermostable DNA polymerase from *Thermus aquaticus* with a non-ionic polymeric detergent (Tween 20) in a storage buffer. The DNA polymerase was described to be useful for improving DNA amplification performed by the PCR technique. In document N28, the president of MBR confirmed that this data sheet was a copy of the handout made freely available by MBR to everyone at the Conference of the American Society of Biological Chemists (ASBC) held on June 7-11, 1987 and distributed to the attendees of this Conference, in which MBR was a registered presenter.

Article 100(a) EPC; Article 56 EPC
Admissibility of documents N33 and N39 into the appeal proceedings

The granted claims were clearly not novel and thus, the inventive step attacks put forward at first instance proceedings were only included for formal reasons. Due to the late filing of the Auxiliary Request containing

the purpose-related feature, there had been no opportunity to file additional documents. Accordingly, the filing of documents dealing with inventive step and with the purpose-related feature were the first possible reaction to the filing of that Auxiliary Request and thus, these documents were *prima facie* relevant and should be admitted into the appeal proceedings.

Document N33, filed with the Grounds of appeal and concerned exclusively with PCR reactions, was relevant in the light of the narrow interpretation made by the opposition division of the purpose-related feature, namely that the thermostable polymerase had to be suitable for a PCR reaction in contrast to a mere nucleic acid amplification. Document N33 was known to the patentee, it was also cited in the Notice of opposition and, although not physically filed, it was clearly identified therein and available to the public in easily accessible electronic (EPO) databases.

Document N39 was filed in reply to the board's communication under Article 15(1) RPBA in which it was noted that there was no document on file disclosing a composition suitable for PCR amplification. Document N39, together with other documents cited in the first instance proceedings and in appeal, proved the suitability of thermostable polymerase compositions for PCR amplification. Document N39 was also cited in the Notice of opposition, in which an attack on inventive step was based on this document. When filing its opposition, the opponent had relied on documents in the public EPO file of the parent case and explicitly

referred to them as "*other evidence*". Accordingly, all these documents were already in the present proceedings.

The problem-solution approach

Document N1, the closest prior art, disclosed a process for purifying the DNA polymerases from the thermophilic *Thermus thermophilus* HB-8. The final fractions of this process, containing pure DNA polymerase and glycerol, retained their activity over a six-month period. There were no contaminating nucleases in these fractions and thus, the pure thermostable DNA polymerase was suitable for PCR amplification. Since it was not clear from document N1 whether these fractions contained the Brij-58 detergent (used in the first process step for lyzing the bacterium cells), the technical difference between the disclosure of document N1 and the composition of claim 1 was the presence of a non-ionic polymeric detergent.

Starting from this prior art, the problem to be solved was the provision of alternative stable compositions comprising a thermostable nucleic acid polymerase for use in a process for amplifying one or more specific nucleic acid sequences using primers. The solution according to the patent-in-suit was to replace the stabilizer glycerol by one or more non-ionic polymeric detergents.

Although there was no direct motivation in document N1 for a skilled person to look for such alternatives, according to the established case law, a skilled person always strived to develop alternatives. Document N31 showed the common general knowledge of a skilled person

at about the first priority date of the patent-in-suit and illustrated that non-ionic polymeric detergents were standard stabilizers used in storage buffers, in particular for enzymes acting on nucleic acids and derived from thermophilic bacteria, and thus, closely resembling the thermostable polymerase disclosed in document N1.

Document N31 also stated that Triton X-100 could break protein hydrophobic aggregates to promote enzymatic activity. As cited in document N4, enzymes derived from thermophilic bacteria had an enhanced thermostability due to the presence of additional hydrophobic interactions. These interactions were known to cause hydrophobic aggregates. Thus, it was obvious for a skilled person to use Triton X-100 as an alternative stabilizer to glycerol for promoting the activity of the thermostable DNA polymerase of document N1. Document N5 disclosed the use of Triton X-100 as an alternative to glycerol for preventing loss of protein and of enzymatic activity by adsorption to surfaces of containers or chromatographic matrices. All this prior art showed that the replacement of glycerol by non-ionic polymeric detergents, in particular Triton X-100, was an obvious alternative to a skilled person. This was supported by further prior art on file showing the use of Triton X-100 with enzymes closely related to that of document N1, such as a DNA polymerase from a Rous Sarcoma Virus (RSV).

XI. The arguments of the patentee, the respondent in these proceedings, may be summarized as follows:

Article 100(a) EPC; Article 54 EPC
Admissibility of documents N27 and N27.2 into the appeal proceedings

The activity of the thermostable polymerase in the composition of granted claim 1 was discussed from the beginning of the opposition proceedings. In line with the definition given in paragraph [0037] of the patent-in-suit, the polymerase was required to be effective for PCR amplification. Neither this activity nor the presence of the detergent Brij-58 was shown for any of the fractions disclosed in document N1, as explicitly stated by the opposition division in its communication attached to the summons to oral proceedings. Nothing prevented the opponent at this point of the proceedings (6 months before the oral proceedings) from filing experimental evidence to support its arguments and convince the opposition division of the contrary.

The amplification reaction was described in the patent-in-suit as having an improved specificity and as resulting in a very distinct signal of the amplified template nucleic acid. Figure 2 of documents N27 and N27.2 did not show a distinct sharp PCR band but only a broad smear band and the degradation of the template nucleic acid to fragments of lower molecular weight by the action of contaminating nucleases present in the sample. Some of these fragments could be used as primers for DNA synthesis. Thus, no amplification - in the sense of the patent-in-suit and of claim 1 - was

shown in Figure 2 of documents N27 and N27.2. There was no positive PCR control in the experiments reported in these documents and the polymerase sample used therein corresponded to intermediate fractions of the purification process of document N1, which were not intended in this document for any particular use, let alone for the use mentioned in claim 1. The results described in Example IX of the patent-in-suit were not comparable to those of documents N27 and N27.2, since they were obtained by using a non-thermostable polymerase with human genomic DNA as a nucleic acid template.

Novelty in the light of documents N1 and N2

The only criterion that had to be satisfied by the enzyme composition of claim 1 was that it had to be suitable and effective for the amplification reaction described in the patent-in-suit, i.e. for PCR amplification. The initial fractions of the purification process of document N1 (cell-lysate, streptomycin treatment) did not show PCR activity nor did documents N27 and N27.2 show it for the dialyzed ammonium sulphate fraction. On the contrary, evidence was on file in which, given the low level of purity of these fractions, one of the authors of document N1 expressed doubts on the presence of such activity and, indeed, even for the final fractions of this purification process.

In cases of documents like document N2, where a prior art disclosure did not enter the public domain by the usual route of publication in a scientific or technical journal, there were established principles in the case

law for assessing whether a disclosure was made available to the public. Applying these principles, document N2, either on its face or in the light of the evidence provided in document N28, had not been made available to the public. The MBR president was not a neutral person attending the ASBC Conference. Whereas he could testify that there was an intention to deliver the MBR leaflet to the public, he could not report what the public actually received. It was doubtful that he was a competent witness 24 years after the event and in absence of any evidence showing that he was actually present at that Conference.

Article 100(a) EPC; Article 56 EPC
Admissibility of documents N33 and N39 into the appeal proceedings

Document N33, cited in the patent-in-suit as background prior art, was not more relevant than document N1. It was clearly derivable from the whole content of the patent, and it was so argued at the beginning of the opposition proceedings, that the activity of the thermostable polymerase had to be suitable for PCR amplification. There was no reason for only filing this document in appeal proceedings, and not earlier in the proceedings.

Likewise document N39 was not more relevant than document N1. Although it was cited in the Notice of opposition, the document was not actually filed in the proceedings. Document N39 was cited and discussed in the parent patent procedure and other prior art documents, declarations and experimental evidence were filed in this procedure for assessing the disclosure of

this document. None of these documents and evidence had previously been filed in the procedure of the present divisional patent application. Thus, if document N39 was admitted at a late stage in appeal proceedings, then all these documents and evidence would also have to be admitted into the appeal proceedings. This would effectively result in the re-opening of the parent patent procedure.

The problem-solution approach

Document N1, the closest prior art document, stated that the final fractions of the purification process, containing a thermostable DNA polymerase and glycerol, retained their activity over a six-month period. Starting from this prior art, the technical problem to be solved was the provision of an alternative composition for stably storing a thermostable nucleic acid polymerase. The claimed subject-matter solved this problem as shown in the examples of the patent-in-suit and in post-published evidence on file. In view of the high stability and satisfactory storage properties reported in document N1, there was no motivation for a skilled person to look for alternative stabilizers. Hindsight was needed to formulate this technical problem and to arrive at the claimed composition.

Both glycerol and bovine serum albumin (BSA) were known as standard stabilizers, the latter also for hydrophobic proteins. They were used in the storage buffers of the enzymes described in document N31, including enzymes isolated from thermophilic bacteria and acting on nucleic acids. There was no reason to look for other alternative stabilizers and, if so, the

prior art would not have led the skilled person to non-ionic polymeric detergents. Whereas Triton X-100 was identified in document N31 as being capable of breaking up hydrophobic aggregates and thereby, to promote enzymatic activity (assay buffer) - which was a different purpose than storage (storage buffer), there was no evidence on file showing that these aggregates were actually formed by the thermostable polymerase of document N1. The presence of extra buried ionic bonds between subunits and additional hydrophobic interactions in proteins derived from thermophilic bacteria referred to in document N4, did not mean that all these proteins formed the aggregates mentioned in document N31. There was no evidence on file suggesting that this was the case for the thermostable polymerase of document N1. Hindsight was needed to derive this information from document N1. In any case, the high stability reported in document N1 did not suggest that there was any problem for the (stable) storage of this polymerase. If, as argued by the appellant, hydrophobic enzymes derived from thermophilic bacteria were normally stored in the presence of these detergents, document N31 showed some hydrophobic enzymes derived from thermophilic bacteria stored without Triton X-100. Moreover, the publication date of document N31 was not unambiguously established.

Although documents N4 and N5 referred to the ability of Triton X-100 to stabilize an enzyme by preventing the loss of its activity during the assay, these documents also warned that Triton X-100 was not beneficial for all enzymes since some enzymes were denaturated. Thus, a skilled person would have been cautious to use it without having further information relating to

thermostable polymerases. However, this information was not present in documents N4 and N5.

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

XIII. The patentee (respondent) requested that the appeal be dismissed.

Reasons for the Decision

Interpretation of claim 1 as maintained by the opposition division

1. The terms "*composition*" and "*comprising*" in claim 1 allow the presence of other products in the composition of claim 1 in addition to the "*thermostable nucleic acid polymerase enzyme*" and "*one or more non-ionic polymeric detergents*". Even if the purpose-related feature in claim 1 is broadly interpreted, the requirement "*for use in a process for amplifying one or more specific nucleic acid sequences ... using primers*" (underlined by the board) is understood by the board to exclude from claim 1 processes in which only a mere replication or a simple synthesis of nucleic acids takes place. In the board's view, the purpose-related feature of claim 1 requires the amplification process to comprise several cycles and these cycles to be specific in the sense described in the patent-in-suit (cf. *inter alia*, page 3, paragraph [0012], lines 5 to 6 of the patent-in-suit).

Article 100(a) EPC; Article 54 EPC

2. Documents N1 and N2 are the only documents cited in the decision under appeal in respect of Article 54 EPC (cf. page 5, point 3.2.1 and page 6, point 3.2.2 of the decision under appeal). In its appeal, however, the appellant has sought to base a case under Article 54 EPC upon the disclosure of documents N27 and N27.2. The admissibility of these documents into the proceedings will now need to be considered.

Admissibility of documents N27 and N27.2 into the appeal proceedings

3. The appellant argues that the granted claims did not comprise any purpose-related feature and thus, the claimed "*stable enzyme composition*" comprising "*a thermostable nucleic acid polymerase enzyme*" was required only to have some polymerase activity. For such subject-matter, it was not necessary to provide any experimental evidence. This evidence only became necessary, at the earliest on 10 February 2011, when the patentee filed an Auxiliary Request containing such a purpose-related feature. However, this Auxiliary Request was filed one month before oral proceedings, and there was not enough time for the opponent to carry out the required experiments (cf. Section X *supra*).

4. While the granted claims did not have a purpose-related feature and, accordingly, the claimed "*stable enzyme composition*" comprising "*a thermostable nucleic acid polymerase enzyme*" could be broadly interpreted, the board notes however that the patentee interpreted this subject-matter in a narrower sense - indeed from the

beginning of the opposition proceedings - and considered that the thermostable polymerase had to be not only active but to have an activity suitable for any assay, in particular, for forming nucleic acid strands complementary to a nucleic acid template strand (cf. page 5, point 1.1.2.5 of the patentee's letter of 26 June 2009 in reply to opponent's Notice of opposition and page 1, last paragraph of the communication of the opposition division dated 24 September 2010 attached to the summons to oral proceedings). The filing of an Auxiliary Request comprising the purpose-related feature on 10 February 2011, in response to the preliminary opinion of the opposition division, made the patentee's interpretation explicit. In view of the course and history of the opposition proceedings, the filing of such a request could have been expected and could not have surprised the opponent (cf. page 18, point 6.6 of the Notice of opposition).

5. Thus, while generally the presence of polymerase activity in the supernatant of both the crude cell-lysate and the streptomycin treated preparation of document N1 was not in doubt (cf. point 14 *infra*), the presence of a polymerase activity in the sense understood by the patentee and as indicated in the patent-in-suit, i.e. suitable for amplifying a specific nucleic acid sequence (cf. *inter alia* page 3, paragraph [0012], page 6, paragraph [0037] of the patent-in-suit), was already disputed from the beginning of the opposition proceedings. Likewise, while the presence of the non-ionic polymeric detergent Brij-58 in the crude cell-lysate was not put into question (cf. point 14 *infra*), the presence of this detergent in other

fractions of the purification method disclosed in document N1 was not considered to be proven but was only presumed from the beginning of the opposition proceedings.

6. As regards the appellant's argument that after the filing of the Auxiliary Request containing the purpose-related feature there was not enough time left for carrying out the required experiments (cf. Section X *supra*), the board notes that the opponent did not request a postponement of the oral proceedings before the opposition division in order to carry out these experiments, nor did it raise "*any objections with respect to the admissibility and allowability of this auxiliary request*" (cf. page 2, point 4.2 of the "Minutes of the oral proceedings before the opposition division", hereinafter "the Minutes"). Since there was no request for correction of these Minutes, they are considered to reflect the essentials of these oral proceedings.

Prima facie relevance of documents N27 and N27.2

7. According to the appellant, the experimental evidence shown in document N27 demonstrated that the *Tth* DNA polymerase(s) present in the ammonium sulphate fraction disclosed in document N1 (*Tth* AS extract) amplified a specific nucleic acid sequence using primers. Moreover said fraction contained the detergent Brij-58. Document N27.2, a copy of improved quality of Figure 2 of document N27, replaced the poor quality electronic reproduction of the original Figure 2 (cf. Section X *supra*).

8. The board concurs with the respondent's view that the experimental evidence in documents N27 and N27.2 is ambiguous and unclear. On the one hand, in the lane of Figure 2A containing the M13 template and the *Tth* AS extract, the intensity of the very high molecular weight band corresponding to the M13 template decreases, which indicates a significant degradation of the template. On the other hand, there is no clear amplification band in the agarose gel of the Southern Blot but rather a broad smear extending from about 50 bp to 150 bp (Figure 2B). A similar smear is also shown in Figure 2A, although a broad band of about 150 bp seems to be identifiable. No positive control with a homogeneous, pure *Tth* DNA polymerase was carried out in these experiments.

9. The absence of a distinct PCR band and the presence of a smear with significant template degradation are the results which have to be expected for a mixture containing several DNA polymerases and contaminating endo- and exonucleases, i.e. presence of (template) degraded fragments and of fragments resulting from a DNA synthesis and/or replication. Although the presence of contaminating nucleases is not explicitly mentioned in document N1, it is clear that they are present in the first purification steps of the method described in document N1 (cf. see, for comparison, page 7, paragraphs [0049] to [0051] of the patent-in-suit). While the wording of claim 1 allows the presence of contaminants in the claimed composition, the purpose-related feature in claim 1 requires that these contaminants do not hinder the amplification of a specific target nucleic acid sequence in the sense of the patent (cf. *inter alia*, page 3, paragraph [0012],

lines 5 to 6 of the patent-in-suit). This is, *prima facie*, not clearly demonstrated in the evidence provided in documents N27 and N27.2.

10. It is true, as pointed out by the appellant, that the patent-in-suit describes the amplification process as yielding a mixture of nucleic acids with the expected target amplified products and various background non target products (cf. page 18, paragraph [0132] of the patent-in-suit). However, this definition is located immediately before a reference to an original DNA template containing multiple target sequences and under the heading "*Amplification Protocol*" in which several conditions and factors are described that may influence the result of the amplification. These are the quality of the target nucleic acid (pure form, complex mixture; page 12, paragraph [0096] of the patent-in-suit), the nature and properties of the primer(s) (specificity, length, amount, collection of primers; page 12, paragraph [0100] of the patent-in-suit) as well as those of the polymerase used (pure form, presence of contaminants and their properties, etc.). In a PCR amplification, none of these factors can produce a background relevant enough to render the amplification reaction non-specific, i.e. without resulting in a distinct signal of amplified nucleic acid (cf. page 3, paragraph [0012] of the patent-in-suit). A distinct band is not present in Figure 2 of documents N27 and N27.2, although the experiments were carried out using a single, pure target nucleic acid sequence and precisely defined primers. Thus, the polymerase sample used in these experiments is considered, *prima facie*, not to be suitable for the amplification required in claim 1.

11. In the board's view, the results reported in Example IX of the patent-in-suit, referred to by the appellant, cannot support its argument that these results are the same as those in documents N27 and N27.2. These results were obtained using samples of human genomic DNA, which is totally different from the pure template used in documents N27 and N27.2. The amplifications that are compared in Example IX are performed by either using a non-thermostable polymerase (Klenow polymerase) or a thermostable (Taq) polymerase. Whereas the former results in a smear of DNA caused by "*... non-specific annealing and extension of primers to unrelated genomic sequences ...* ", the latter is said to be highly specific and to result in greater yields of the target sequence (cf. page 32, paragraphs [0264] and [0265] of the patent-in-suit). Indeed, these are the results expected by a skilled person for an amplification using a thermostable polymerase as disclosed in the patent-in-suit, namely the presence of a distinct sharp (PCR) band which is not shown in Figure 2 of documents N27 and N27.2.

Conclusion on the admissibility of documents N27 and N27.2

12. Thus, the board considers that the experimental evidence of documents N27 and N27.2 is *prima facie* of no relevance. Therefore, in exercising its discretion pursuant to Article 12(4) RPBA, the board decides not to admit these documents into the appeal proceedings.

Novelty in the light of documents N1 and N2

13. Turning now to the case on novelty based on the disclosure of documents N1 and N2, it is first of all necessary to examine the disclosure of these documents.

14. Document N1 discloses the purification of three DNA polymerase isoenzymes from *Thermus thermophilus* (*Tth*) HB-8. In a first step, frozen cells were thawed and suspended in a buffer containing 20% glycerol (buffer A). The cells were lysed by adding a solution containing, among other compounds, the non-ionic polymeric detergent Brij-58. The final suspension was centrifuged and the supernatant fraction treated with streptomycin sulphate and then centrifuged to remove the nucleic acids. Solid ammonium sulphate was added and the suspension stirred and centrifuged. The resulting pellets were resuspended in buffer A (cf. page 42, right-hand column under the heading "*Purification of DNA polymerases*" of document N1). In the Notice of opposition, the supernatants of the crude cell-lysate and of the treated streptomycin preparation were identified as containing a thermostable nucleic acid polymerase in a buffer comprising a non-ionic detergent (cf. page 4, point 5.1.1 to page 6, point 5.1.3 of the "Notice of opposition"). In its reply to the communication of the opposition division accompanying the summons to oral proceedings, the opponent referred to the thermostable *Tth* DNA polymerases obtained from further purification steps and to the presumption that the Brij-58 detergent persisted through these steps, although emphasis was put on the crude lysate (cf. page 2, point A3 and page 3, point A5 of opponent's letter of 9 February 2011).

15. In the decision under appeal, the opposition division considered that it could not "*... be concluded that the supernatant obtained in N1 can be used in a PCR reaction i.e. the amplification of a specific fragment. It is uncertain if the amount of enzyme in this lysate is sufficient to carry out the reaction. Furthermore, the presence of nucleases and proteases in said lysate clearly disturb the specific amplification of nucleic acid fragments. To which extend, however, remains unknown. Therefore, in the absence of a corresponding prove ... reasonable doubts remain*". Accordingly, document N1 was considered not to anticipate the claimed subject-matter (cf. page 6, first paragraph of the appealed decision). With its Grounds of appeal, the appellant filed document N27 which contained experimental evidence in support of its argument of lack of novelty based on document N1. As set out above, the board has decided not to admit documents N27 and N27.2 into the proceedings.

16. Document N2 is a product information data sheet of Molecular Biology Resources, Inc. (MBR) which discloses the thermostable DNA polymerase of *Thermus aquaticus* (Taq polymerase) in a storage buffer containing a non-ionic polymeric detergent (Tween 20). The appellant argues that document N2 was distributed on 8 June 1987 at the Conference of the American Society of Biological Chemists (ASBC). In the decision under appeal, the opposition division considered the following three questions, namely i) had N2 been made available at the meeting by distribution?, ii) was there a prior use?, and iii) was it, if document N2 was indeed available, a non-prejudicial disclosure in the sense of

Article 55(1)(a) EPC? (cf. page 7 of the decision under appeal). After a thorough consideration, the opposition division decided all three questions in the patentee's favour and it was concluded that document N2 did not belong to the state of the art (cf. page 12, first paragraph of the decision under appeal).

17. In its grounds of appeal, the appellant filed document N28, a declaration of the President and CEO of MBR, Dr Peter J. Smyczek, in which he stated that document N2 was distributed and made available to everyone freely at the ASBC Conference held on 7-11 June 1987. In the communication under Article 15(1) RPBA, the board referred to the criteria established by the Boards of Appeal for assessing oral and public disclosures (cf. "Case Law of the Boards of Appeal of the EPO", 6th edition 2010, I.C.1.8, page 69, in particular I.C.1.8.3, page 73) and questioned whether these criteria were fulfilled by document N28. No further comments in this respect were made by the appellant in its reply to this communication and at the oral proceedings before the board. Instead, the appellant referred to its written submissions. Without entering into thorough considerations about the admissibility of document N28 into the appeal proceedings, suffice it to say that the deficiencies outlined in the board's communication have not been overcome. In particular, as pointed out by the respondent, the president of MBR cannot be seen as a neutral attendee of that Conference. Moreover, there is no evidence on file showing that the president of MBR actually attended the Conference and supervised the actual distribution of the data sheet or, as mentioned in the decision under appeal, who were the actual

recipients of this data sheet. In view of these factors, document N28 is considered not to be of further relevance. As stated in the communication under Article 15(1) RPBA, there is no reason to deviate from the findings of the opposition division as regards the public availability and the disclosure of document N2.

Conclusion on novelty

18. There is no evidence on file demonstrating, in a clear and unambiguous manner, that any of the fractions obtained in the purification method disclosed in document N1, in particular the crude cell-lysate, the streptomycin treated preparation and the *Tth* AS extract, are suitable for the purpose indicated in claim 1. Moreover, there is no evidence on file showing the presence of the detergent Brij-58 in other fractions than the AS extract. Thus, in line with the decision under appeal, document N1 is considered not to anticipate the subject-matter of claim 1. Moreover, as stated in point 17 *supra*, there is no reason to deviate from the findings of the opposition division as regards document N2.

19. Thus, the requirements of Article 54 EPC are fulfilled.

Article 100(a) EPC; Article 56 EPC

20. In the appealed decision, document N1 is identified as the closest prior art and no other documents are cited as possible alternatives (cf. page 14, penultimate paragraph of the decision under appeal). In its Grounds of appeal and in reply to the board's communication under Article 15(1) RPBA, the appellant cited several

other documents as alternative closest prior art. At the oral proceedings before the board, only documents N33 and N39 were cited as possible alternative closest prior art. Since none of these documents is cited in the decision under appeal, the question arises whether they are admissible into the appeal proceedings. There is no need to consider the admissibility of other documents not cited at these oral proceedings.

Admissibility of documents N33 and N39 in appeal proceedings

21. Document N33 was filed with appellant's Grounds of appeal and identified therein as a "new" document (cf. page 2, point B.1 of appellant's Grounds of appeal). This document was mentioned as general background prior art with reference to the earlier parent patent in the Notice of opposition, where no particular passage thereof was referred to and where it was not elaborated that it was of relevance in the formulation of the problem-solution approach (cf. page 14, penultimate paragraph of the Notice of opposition). The document was also cited in the context of a hypothetical claim directed to a PCR kit composition (cf. page 18, point 6.6 in the Notice of opposition). Document N33 was not physically filed nor incorporated in the list of documents presented as evidence for the opposition. The document was neither cited in the communication of the opposition division attached to the summons to oral proceedings nor in the Minutes of these oral proceedings or in the decision under appeal.

22. The appellant justified the late filing of document N33 in its statement of the Grounds of appeal by arguing that its relevance became only evident after the filing

of an Auxiliary Request with the purpose-related feature. However, as stated in point 4 *supra*, a narrow interpretation of the DNA polymerase activity was already an issue at the beginning of the opposition proceedings. Already at that point in time, the appellant was free to formulate a problem-solution approach based on document N33 and, this was certainly true, after the filing of the Auxiliary Request containing the purpose-related feature, one month before the oral proceedings at first instance. Document N33 was known to both parties as can be seen from its citation in the appellant's Notice of opposition and from the references in the patent-in-suit (cf. page 2, paragraph [0006] and page 12, paragraph [0093] of the patent).

23. In the light of these considerations, the board takes the view that document N33 could have been filed in the first instance proceedings. To admit it now into the appeal proceedings, would amount to the opening of a fresh case which is contrary to the purpose of appeal proceedings (cf. "Case Law", *supra*, VII.E.1, page 821). It is also to be noted that the patent-in-suit is a divisional application of an earlier patent application which, upon grant, was opposed and the decision of the opposition division subsequently appealed. This appeal led to a decision by this board in a different composition (cf. decision T 1080/01 of 24 October 2003 cited in appellant's Notice of opposition). Thus, the patent resulting from the parent application underwent a full examination by both the first and appeal instances of the EPO. Therefore, the board in exercising its discretion pursuant to Article 12(4)

RPBA decides not to admit document N33 into the appeal proceedings.

24. Document N39 was filed by the appellant in reply to the board's communication under Article 15(1) RPBA and thus, it represents an amendment to the appellant's case in the sense of Article 13(1) RPBA. The document was cited in the appellant's Notice of opposition by quoting *expressis verbis* the decision of the opposition division on inventive step of the earlier parent patent (cf. page 10, point 6.1 and 6.1.1 of the Notice of opposition). Document N39 was not physically filed nor incorporated in the list of documents presented as evidence for the opposition. Apart from this reference, the appellant's attack under Article 56 EPC in the Notice of opposition was essentially based on document N1 as closest prior art document (cf. page 15, point 6.3 and page 17, point 6.4 of the Notice of opposition). In harmony with this approach, the patentee's reply on 26 June 2009, although mentioning document N39, referred to and dealt in detail only with document N1 as the closest prior art document. Likewise, the communication of the opposition division attached to the summons to oral proceedings referred only to document N1. This is also evident from the decision under appeal and in the Minutes, wherein only document N1 is cited as the closest prior art document (cf. page 14, penultimate paragraph of the decision under appeal).

25. The reasons given by the appellant for the introduction of document N39 at this stage of the appeal proceedings are basically those given for the admission of document N33 (cf. point 22 *supra*). In addition, document N39 was also known to the parties from the beginning of the

opposition proceedings. The board decided not to admit document N33 and sees no reason not to reach the same decision as regards document N39. Thus, in exercising its discretion pursuant to Article 13(1) RPBA, the board decides not to admit document N39 into the appeal proceedings.

26. It is worth noting here that, according to the established case law, the procedure concerning a divisional application is completely independent from that of the parent application, the former being examined entirely separately from the latter (cf. "Case Law", *supra*, IV.1.1, page 375 and IV.7.1, page 391). Facts, evidence and requests or submissions made or filed in the parent procedure are not automatically part of the divisional procedure. A general citation or a mere reference to facts and/or evidence, such as to prior art documents, filed in the parent procedure but not physically filed or incorporated into the divisional application procedure does not constitute a reservoir upon which a party may draw at its convenience and at any time in the divisional application procedure.

The problem-solution approach

27. From the prior art documents filed in opposition proceedings (documents N1 to N21), document N1 has been identified as representing the closest prior art document in the appealed decision and at the oral proceedings before the board. The disclosure of this document has already been discussed in point 14 *supra*. It is worth noting here that the final fractions resulting from the purification process were dialysed

against buffer A, a buffer containing the known enzyme stabilizer glycerol (cf. page 42, right-hand column under the heading "*Purification of DNA polymerases*"), and that at the end of this process "*... the enzymes are stable for one or two weeks at 20°C. The final fractions have retained their activity over a six-month period stored at -20°C, ...*" (cf. page 44, left-hand column, first paragraph).

28. Starting from this prior art, the technical problem to be solved has been formulated as "*... the provision of a stabilized thermostable polymerase composition ...*" (cf. page 14, penultimate paragraph of the decision under appeal) or "*... the provision of an alternative composition for stably storing thermostable nucleic acid polymerase ...*" (cf. page 25, point 5.3.4 of respondent's letter of 21 March 2012 in reply to appellant's Grounds of appeal). The same problem has also been formulated in the appellant's Grounds of appeal, although starting from different prior art documents (cf. page 20, paragraph [100] and page 24, paragraph [128] of appellant's Grounds of appeal). As a solution to this problem, the patent-in-suit proposes the composition according to claim 1 characterized by the presence of non-ionic polymeric detergents. The board is convinced that the technical problem is solved and the appellant has not argued otherwise.

29. The board agrees with the respondent that there is no motivation for a skilled person in document N1 to look for an alternative stabilized thermostable nucleic acid polymerase composition or, in other words, to look for a stabilizer other than glycerol. There is no explicit reference thereto in document N1 nor is the reported

stability of the stored final fractions so low as to prompt a skilled person to look for such alternatives.

30. As regards obviousness, the disclosure of documents N4 and N5 in combination with those of document N1 has been raised. Documents N4 and N5, which are part of a general textbook of enzymology, are concerned with the structure and stability of proteins in general and with the activity of enzymes. Under the heading "*Stabilization*" the reader's attention is drawn to the fact that "*... loss or denaturation of proteins by adsorption onto glass, quartz, plastic, cellulose, or dialysis membrane, can be prevented in several ways ...*" and that, for "*... glass and plastic surfaces ... modifying the solvent is more effective in preventing protein losses than modifying the container surface ...*", wherein "*(t)he solvent is modified by adding either 50% glycerol, 0.2 mM Triton X-100, or 0.1 mg/mL bovine serum albumin ...*" (cf. page 17, lines 12 to 14 and paragraph bridging pages 17 and 18 of document N4). In order to prevent the adsorption of the enzyme into the surface of the reaction vessel, it is necessary that "*(b)ovine serum albumin (1 mg/mL), 20% glycerol (v/v), or 2mM Triton X-100 may be included in the assay ... in an attempt to stabilize the enzyme ...*" (cf. paragraph bridging pages 59 and 60 of document N5). Thus, these documents disclose the use of glycerol, Triton X-100 or BSA, as possible alternatives to prevent protein loss by adsorption to certain surfaces of containers (cf. page 126, last full paragraph of document N5).

31. Under the same heading, it is described that "*(s)everal protein modifications ... prevent denaturation of*

proteins" and, as alternative, "*... the desired enzyme can be isolated from a thermophilic organism ...*" (cf. paragraph bridging pages 16 and 17 of document N4). Indeed, when describing the effects of temperature on enzymes, proteins from thermophiles are described as usually withstanding elevated temperatures since "*(e)xtra buried ionic bonds (salt bridges) between subunits and additional hydrophobic interactions amongst loops of the polypeptide chain at the core of multimeric thermophilic proteins ... account for the enhanced thermostability. Additional hydrophobic interactions at the edge of the subunit interfaces may also prevent access of water to the interior of the protein molecule*" (cf. page 11, last paragraph and page 12, first paragraph of document N4). These disclosures imply, as the opposition division has pointed out, that enzymes from thermophiles are inherently stable (cf. page 14, lines 9 to 11 of the appealed decision).

32. Under the heading "*Denaturation*", document N4 discloses that, whereas "*(i)onic detergents are particularly effective protein denaturants ... (n)on-ionic detergents like Triton X-100, on the other hand, do not bind to proteins as the ionic ones do. Most, but not all, enzymes retain complete catalytic activity in Triton X-100 ...*" (underlined by the board) (cf. page 15, last paragraph of document N4). Thus, whereas Triton X-100 may be used to prevent the loss of protein by adsorption to certain surfaces, at least for some enzymes, its use may result in their denaturation.

33. Thus, the skilled person, knowing that enzymes from thermophiles were inherently stable and that several stabilizers - among them the well-known, standard

glycerol used in document N1 - were suggested in documents N4 and N5, in the light of the high stability reported in document N1 (cf. point 27 *supra*), would have had no reason to contemplate the replacement of glycerol by Triton X-100 in the final fractions disclosed in document N1. *A fortiori*, the skilled person would not expect to achieve any advantage by such a replacement. On the contrary, according to documents N4 and N5, a certain risk of worsening the stability results reported in document N1 due to denaturation of the enzyme could not be completely disregarded.

34. Thus, even accepting that a skilled person always strives to develop alternatives and to look for ways of improving on known techniques and products, the board is convinced that the replacement of glycerol by a non-ionic polymeric detergent is not derivable in an obvious manner from the prior art on file, in particular, not from documents N4 or N5.

35. The board further agrees with the opposition division that other prior art documents cited in the opposition proceedings could not have changed the attitude of the skilled person (cf. paragraph bridging pages 13 and 14 of the decision under appeal). Although non-ionic detergents are cited in these documents, these detergents are used for purposes other than storage stability, such as promotion of cell-lysis and prevention of non-specific adsorption in chromatographic columns. Moreover, these documents are concerned with non-thermostable polymerases or with thermostable enzymes other than polymerases and which have physical and structural properties different from

those of thermostable polymerases. It is also noted that, for storage purposes and stability studies, reference is made in several of these documents to glycerol.

36. Document N31, a new document filed with the appellant's Grounds of appeal, shows the presence of non-ionic polymeric detergents in the storage buffer of several enzymes. However, although some of them are thermostable and/or have nucleic acid as a substrate, none of them is a thermostable nucleic acid polymerase. The presence of glycerol and/or BSA in the storage buffer of other enzymes is also disclosed in document N31. The detergent Triton X-100 is described to promote enzymatic activity by breaking up hydrophobic aggregates of protein - which is a different purpose than storage stability, but there is no evidence that such aggregates are formed by thermostable polymerases, in particular not by the DNA polymerase disclosed in document N1. Most importantly, document N31 is an extract compilation of several commercial molecular biology catalogues for which the exact publication dates have not been provided. Some of these catalogues refer to 1986/1987, 1987 and even 1987/1988, which would be later than the claimed first priority date (22 August 1986). No reasons have been given to explain why this document was not filed at the first instance proceedings but only at this late stage of the proceedings. In view of its substantial deficiencies, the board considers that a discussion on the formal admissibility of this document into the appeal proceedings is not necessary.

37. In view of the conclusions reached above, namely that there is no evidence on file rendering obvious a stable enzyme composition of a thermostable polymerase and one or more non-ionic polymeric detergent, the board does not see any need to consider in detail whether the thermostable DNA polymerase of the final fractions disclosed in document N1 is suitable for amplifying one or more specific nucleic acid sequences using primers. The same applies for other thermostable polymerases disclosed in documents cited and discussed in the course of the prosecution of the parent application. These documents were not explicitly introduced into the first instance proceedings from which the current appeal derives and their admissibility into the present appeal procedure has not been considered since the appellant limited his case to the documents cited in this decision.

Conclusion on inventive step

38. In view of the above considerations, the board does not see any reason to deviate from the decision under appeal as regards Article 56 EPC and considers that the claimed subject-matter fulfils the requirements of this Article.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski M. Wieser