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D E C I S I O N
of 30 October 2003

Case Number: T 0078/96 - 3.3.4

Application Number: 86302299.2

Publication Number: 0201184

IPC: C12P 19/34

Language of the proceedings: EN

Title of invention:
Process for amplifying nucleic acid sequences

Patentee:
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Opponents:
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Abbott Laboratories
Pasteur Sanofi Diagnostics
Rhône Poulenc Agriculture Limited

Headword:
PCR/Hoffmann-La Roche

Relevant legal provisions:
EPC Art. 54, 56

Keyword:
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
T 0838/97, T 0330/92, T 1121/97, T 0206/83, T 0902/94

Catchword:
-

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Case Number: T 0078/96 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 30 October 2003

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Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted 14 December 1995 concerning maintenance of European patent No. 0201184 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: R. E. Gramaglia
S. C. Perryman

Summary of Facts and Submissions

I. European Patent No. 0 201 184 (application No. 86 302 299.2) claiming priorities from US 716975 of 28 March 1985 (P1) and US 791308 of 25 October 1985 (P2) was filed on 27 March 1986. The patent relates to a process for amplifying nucleic acid sequences (now known as polymerase chain reaction (PCR)) and was granted on the basis of 18 claims.

II. Notices of opposition were filed by six opponents (01) to (06) all requesting the revocation of the European patent on the grounds of Article 100(a), (b) and (c) EPC. By a decision posted on 14 December 1995, the opposition division held that the claims of the auxiliary request filed during oral proceedings satisfied the requirements of the EPC. Claims 1 and 2 of this request read as follows:

"1. A process for exponentially amplifying at least one specific double-stranded nucleic acid sequence contained in a nucleic acid or a mixture of nucleic acids, wherein each nucleic acid consists of two complementary strands, of equal or unequal length, and wherein the sequence to be amplified is contained within a larger sequence, which process comprises:

(a) treating the strands with a molar excess of two oligonucleotide primers, one for each of the strands, under hybridizing conditions and in the presence of an inducing agent for polymerization and the different nucleotides, such that for each strand an extension product of the respective primer is synthesized which is complementary to the nucleic acid strand, wherein

said primers are selected so that each is substantially complementary to one end of the sequence to be amplified on one of the strands such that an extension product can be synthesized from one primer, which, when it is separated from its complement, can serve as a template for synthesis of an extension product of the other primer;

(b) separating the primer extension products from the templates on which they were synthesized to produce single-stranded molecules;

(c) treating the single-stranded molecules generated from step (b) with the primers of step (a) under hybridizing conditions and in the presence of an inducing agent for polymerization and the different nucleotides such that a primer extension product is synthesized using each of the single strands produced in step (b) as a template; and if desired,

(d) repeating steps (b) and (c) at least once; whereby the amount of the sequence to be amplified increases exponentially relative to the number of steps in which primer extension products are synthesized.

2. A process for exponentially amplifying at least one specific double-stranded nucleic acid sequence contained in a nucleic acid or a mixture of nucleic acids wherein each nucleic acid consists of two complementary strands, of equal or unequal length, which process comprises:

(a) treating the strands with a molar excess of two oligonucleotide primers, one for each of the strands,

under hybridizing conditions and in the presence of an inducing agent for polymerization and the different nucleotides, such that for each strand an extension product of the respective primer is synthesized which is complementary to the nucleic acid strand, wherein said primers are selected so that each is substantially complementary to one end of the sequence to be amplified on one of the strands such that an extension product can be synthesized from one primer which, when it is separated from its complement, can serve as a template for synthesis of an extension product of the other primer;

(b) separating the primer extension products from the templates on which they were synthesized to produce single-stranded molecules;

(c) treating the single-stranded molecules generated from step (b) with the primers of step (a) under hybridizing conditions and in the presence of an inducing agent for polymerization and the different nucleotides such that a primer extension product is synthesized using each of the single strands produced in step (b) as a template; and

(d) repeating steps (b) and (c) at least three times; whereby the amount of the sequence to be amplified increases exponentially relative to the number of steps in which primer extension products are synthesized."

Claims 3 to 17 were addressed to specific embodiments of the process of claims 1 or 2. Claims 18 and 19 were directed to the use of a pair of oligonucleotide primers for the amplification of a pre-selected

specific nucleic acid sequence by a process as defined in any one of claims 1 to 17.

III. Appellants I, II and VI (opponents (01), (02) and (06)) filed appeals against the decision of the opposition division. In a communication of 23 July 1996, the board expressed its provisional opinion about the admissibility of these appeals.

IV. Appellant I withdrew the appeal.

V. All the parties except for the respondent announced they would not attend oral proceedings.

VI. The following documents are cited in the present decision:

(D3) NIH Grant Application filed by Prof. H.G. Khorana on 21 October 1969;

(D4) Kleppe K. et al., J. Mol. Biol., Vol. 56, pages 341-361 (1971);

(D6) Khorana H.G. et al., J. Mol. Biol., Vol. 72, pages 209-217 (1972);

(D7) Research Proposal (period from 1 February 1973 to 31 January 1978) submitted by Prof. H.G. Khorana at the National Science Foundation;

(D9) Panet A. et al., J. Biol. Chem., Vol. 249, pages 5213-5221 (1974);

(D21) Suggs S.V. et al, Proc. Natl. Acad. Sci. USA, Vol. 78, pages 6613-6617 (1981);

(D40) Itakura K. et al., J. Am. Chem. Soc., Vol. 97, No. 25, pages 7327-7332 (1975);

(D41) Katagiri N. et al., J. Am. Chem. Soc., Vol. 97, No. 25, pages 7332-7337 (1975);

(D42) Sood A.K. et al., Nucleic Acids Research, Vol. 4, No. 8, pages 2757-2765 (1977);

(D43) Gillam G. et al., J. Biol. Chem., Vol. 253, pages 2532-2539 (1978);

(D47) Ryan M.J. et al., J. Biol. Chem., Vol. 254, pages 5817-5826 (1979);

(D48) Manuscript of a lecture given by Dr K. Kleppe at the Gordon Research Conference on 18 June 1969;

(D63) Declaration of Dr R. Kleppe Aakvaag dated 14 February 1995;

(D70) Declaration of F.A. Faloona dated 10 February 1995;

(D70.1) K.B. Mullis et al., Meth. Enzymol., Vol. 155, pages 335-350 (1987);

(D70.2) K.B. Mullis et al. in "The Polymerase Chain Reaction", Birkhäuser, Boston, Basel, Berlin, pages 430-432;

(D70.3) Dr K.B. Mullis Testimony in the case Hoffmann-La Roche vs Promega Corporation, pages 31-33, 86-88 and 116;

(D72) Hong G.F., Bioscience Report, Vol. 1, pages 243-252 (1981);

(D78) Declaration of Prof. Sir Aaron Klug before the USPTO dated 31 May 1990;

(D79) Declaration of Dr T.J. White dated 31 May 1990.

VII. The written submissions by the appellants and the other parties can be summarized as follows:

Novelty
Oral disclosure by Dr Mullis

– According to document (D70), the claimed subject-matter had been made available to the public before the earliest priority date of the patent in suit by Dr Mullis, who talked about the PCR "concept" publicly outside the Cetus company, his employer. The facts mentioned in document (D70) were confirmed by document (D70.2) and by the Dr Mullis Testimony (document (D70.3)).

Oral disclosure by Dr Kleppe

– The claims at issue lacked novelty in view of a lecture given on 18 June 1969 by Dr K. Kleppe at the Gordon Research Conference. Figure 10 of the

manuscript of the lecture (see document (D48)) showed in schematic form the PCR technique up to the first cycle of "repair replication" yielding two duplexes starting from one single duplex. Figure 11 thereof related to a diagram (cpm vs time) of the incorporation of ^{14}C-dCTP into "duplex II". It could be deduced without any doubt from this figure that exponential amplification of the DNA duplex actually took place during the "repair replication" (see also paragraph 12 of document (D63)).

Documents (D3) and (D7)

– The claims at issue lacked novelty in view of both documents (D3) (see page 37) and (D7) (see page 18), which presented reaction schemes for the replication of the gene corresponding to alanine tRNA using DNA polymerase and the four deoxynucleotide triphosphates. It was made clear that the primers should be antiparallel and hybridise to opposite ends of the double stranded material to be replicated. The overall method for replication of a double-stranded DNA had to involve the following steps: (1) denature the bihelical structure in the presence of an excess of the two appropriate primers; (2) repair in the presence of the enzyme and the four deoxynucleotide triphosphates so as to complete the "doubling" of the original duplex; (3) repeat denaturation and the whole cycle.

Document (D4)

– This document, like documents (D3), (D6), (D7) and (D9) originated from a team headed by Prof. Khorana at the Institute for Enzyme Research of the University of Wisconsin, Madison. In the last paragraph on page 360 of this document, the entire operating procedure of the PCR technique was clearly set out. This process began with a step of heating a DNA duplex to separate the strands. Upon cooling in the presence of an excess of two appropriate primers, two primer-template complexes formed. Repair replication in the presence of DNA polymerase and the four deoxynucleotide triphosphates (dCTP, dTTP, dGTP and dATP) yielded two copies of the original DNA duplex. Repetition ad libitum of the entire cycle described above resulted in exponential amplification (PCR) as claimed.

Document (D6)

– The claims at issue lacked novelty in view of page 211, last paragraph of document (D6), wherein there was stated:

"The duplex could be subjected to a repair reaction by the DNA polymerase of Escherichia coli, the repaired strands separated and the separated strands could again be annealed with a partly complementary polydeoxynucleotide and the repair reaction could be repeated".

Document (D9)

– Reference was made on page 5220, r-h column, first paragraph of document (D9), another article from the team headed by Prof. Khorana, to unpublished work performed by Dr Molineux pertaining to the replication of relatively short DNA duplexes by means of a repair replication process according to documents (D3), (D4) or (D7). These experiments showed *inter alia* that "[(b)] to form the appropriate primer-templates complexes, it was necessary to heat and cool in the presence of an excess (10 times or more) of the appropriate primers and [(c)] in order to perform multiple cycles of repair replication, it was necessary to add, after each cycle, fresh amounts of the primers so as to maintain the appropriate primer-template ratios". From this document it could be concluded that Dr Molineux successfully put into practice the claimed amplification technique.

Oligonucleotide primers

– While the above references were enabling for performing the repair replication technique at their publication date, the Prof. Khorana group did not consider the provision of many replications of practical utility, so that no attempt to carry out eg five or ten cycles of PCR took place. This was because, as shown by Dr Molineux (see preceding paragraph), this technique required a high quantity of oligonucleotide primers. However, oligonucleotides primers, whose synthesis required several months and sometimes years, were scarce. Furthermore, other ancillary technologies such as DNA

sequencing and automated oligonucleotide synthesis, had still to be discovered. This, however, was the only reason for not going ahead according to this concept which actually reflected exactly what is claimed in the patent in suit.

Inventive step

Claim 1

– Even if claim 1 required that the sequence to be amplified had to be contained within a larger sequence, it was obvious to the skilled person that the sequence being amplified by the repair reparation technique disclosed by document (D4) could be contained in a larger sequence. For instance, it was already known to use oligonucleotide primers to "mark" the beginning and/or the end of a sequence of interest (see document (D72)).

Claim 2

– The question of inventive step has to be addressed from the viewpoint of the skilled person in March 1985, ie the filing date of priority document (P1) underlying the patent in suit. By that time, there had been considerable advances in term of the availability of oligonucleotide primers, owing to the development of ancillary technologies such as automated oligonucleotide synthesis. The hybridizing conditions were well known in March 1985. Therefore, a PCR process with an increased number of amplification steps was within the competence of the skilled person.

– Even if documents (D4), (D3), (D6), (D7) and (D9) or the oral disclosure by Dr Mullis (see documents (D70), (D70.2) and (D70.3)) lacked some technical information as to how to carry out the PCR process, the skilled person would have easily arrived at the process of claim 2 by optimising the disclosed technique in the light of the common general knowledge in March 1985.

VIII. The submissions in writing by the respondent can be summarized as follows:

Novelty
Oral disclosure by Dr Mullis

– As regards any of the oral disclosures by Dr Mullis (referred to in document (D70)), it could not be deduced from document (D70) that the persons Dr Mullis talked to were not obliged to confidentiality and that a sufficient amount of details were revealed so as to provide an enabling disclosure.

Oral disclosure by Dr Kleppe

– The oral presentation made by Dr K. Kleppe, of which document (D48) was a manuscript, did not belong to the prior art in accordance with the rationale of decision T 838/97 of 14 November 2000 that disclosures made at a Gordon Research Conference did not form prior art. If anything, Figure 11 of document (D48) showed that Dr K. Kleppe merely achieved linear rather than

exponential amplification, since the figure showed only 8,000 cpm of incorporated ^{14}C-dCTP instead of the 16,000 cpm expected for an exponential amplification.

Claim 1

– Claim 1 required that the sequence to be amplified had to be contained within a larger sequence. No prior art disclosed this feature.

Claim 2
Documents (D3), (D4), (D6), (D7) and (D9)

– Neither NIH Grant Application (D3) nor Research Proposal (D7) were publicly available.

– Even if documents (D3) and (D7) formed prior art pursuant to Article 54(2) EPC, neither these nor publicly available documents (D4), (D6) and (D9) provided sufficient information enabling the skilled person to carry out exponential nucleic acid amplification requiring at least five cycles of amplification.

– Claim 2 related to a process for exponential amplification which required at least five cycles of amplification. There was no such exponential amplification with at least five cycles of amplification in the prior art.

– As for document (D4), the group headed by Prof. Khorana never published the successful performance of the hypothetical process disclosed

in the last paragraph on page 360 of this document. The latter process differed from the method of claim 2 by the fact that the extension product obtained by repair replication was incomplete.

– Page 211, last paragraph of document (D6) merely related to a hypothetical process.

– All the prior art documents (D4), (D6) and (D9) did not provide any experimental conditions required for nucleic acid exponential amplification. These were to be found in the patent in suit only (see page 6, lines 21 to 33 and Examples 1 to 10 and 12).

Oligonucleotide primers

– All the materials required for carrying out exponential amplification, including the oligonucleotide primers, had been available long before the earliest priority date of the patent in suit (see documents (D40) to (D43)). Therefore, the reason for which the Prof. Khorana's group was not able to perform repeated repair replication could not be ascribed to the lack of oligonucleotide primers, but rather to the fact that these scientists were merely looking for a method for increasing the amount of their synthetic, complete tRNA genes and never contemplated exponential multiplication of trace quantities of DNAs.

Inventive step

Claim 1

– Even if documents (D3), (D7), the lecture given by Dr K. Kleppe (document (D48)) and the oral disclosure by Dr Mullis referred to in document (D70) formed prior art pursuant to Article 54(2) EPC, like documents (D4), (D6) and (D9), all this prior art was only concerned with the unsuccessful attempt to amplify a restricted number of gene fragments. Therefore, the skilled person would not have rendered more complex an experiment that already did not work in its simpler version, by turning to a sequence being amplified contained within a larger sequence instead of an entire sequence. The skilled person would also not have combined document (D4) with document (D72), as the latter was concerned with a completely different field (DNA sequencing).

Claim 2

– The closest prior art was represented by the hypothetical statement (see point 3 below for further details) in the last paragraph on page 360 of document (D4). It was not obvious to try to modify this hypothetical statement so as to turn it into the exponential amplification as claimed.

– There was no reasonable expectation of success in arriving at the method of claim 2. The skilled person had to enter unexplored and unpredictable areas. Given that the team headed by Prof. Khorana had been unable to carry out cycled repair reparation, a series of true obstacles had to be overcome by extensive and systematic

experimentation (see document (D75), paragraphs 28 and 29; document (D78), paragraph 14 and document (D79), paragraph 13).

IX. The appellants (opponents (02) and (06)) requested that the decision under appeal be set aside and that the patent be revoked.

The respondent (patentee) requested that the appeals be dismissed.

Reasons for the decision

1. The appeals by opponents (02) and (06) (appellants II and VI, respectively) are admissible.

Novelty (Article 54 EPC)
Oral disclosure by Dr Mullis

2. Relying on documents (D70), (D70.2) and (D70.3) the appellants argue that Dr Mullis, the named inventor, orally disclosed the invention prior to the priority date to a member of the public. Document (D70) is a declaration made by a Dr Faloona, the assistant and fellow employee of Dr Mullis at Cetus Corporation, that he was present at meetings of Dr Mullis with scientists not employed by Cetus to whom Dr Mullis is supposed to have described his invention in detail and the progress of the experiments. Discussions took place in particular with a Dr Ronald Cook.

3. Document (D70.2) are extracts of a post-published book on PCR, one of the authors of which was Dr Mullis.

Statements appear here in the first person that before the priority date Dr Mullis discussed with Dr Ron Cook and others the invention, and that Dr Ron Cook was the only one who shared his enthusiasm for the reaction.

4. Document (D70.3) is part of a deposition made in US court proceedings between Hoffman-La Roche, Inc et al (apparently the assignees of the US as well as the European patent in suit) and a licensee. Dr Mullis confirms that he discussed the idea with Dr Cook, and with others. The context is indicated by the statement "I didn't want to announce it publicly in a forum where there were people, but I didn't mind telling Mickey" whom he had previously described as a real good friend whom he bounced things off.

5. None of this represents evidence of precisely what was said, to whom, or when, or that the recipients even thought that they were free to use or disseminate this information. This evidence does not convince the board that anything was made available to the public by the inventor which can be used as prior art to attack the patent in suit.

Document (D4)

6. Document (D4) is concerned with repairing in vitro synthesized, incomplete duplex DNA portions corresponding to parts of the gene for the yeast alanine tRNA, where one of the strands in the duplex is shorter than the other. The authors (Prof. Khorana's group) show that in the presence of a DNA polymerase and the four deoxynucleotide triphosphates, the longer strand acts as a template and the shorter one acts as a

primer so that the shorter one is elongated (in direction 5' 3') and the incomplete duplex becomes a fully double-stranded DNA molecule. Radioactive nucleotides are used as means to follow the elongation reaction. At the end of document (4), the following statement is made:

"The principles for extensive synthesis of the duplexed tRNA genes which emerge from the present work are the following. The DNA duplex would be denatured to form single strands, This denaturation step would be carried out in the presence of a sufficiently large excess of the two appropriate primers. Upon cooling, one would hope to obtain two structures, each containing the full length of the template strand appropriately complexed with the primer. DNA polymerase will be added to complete the process of repair replication. Two molecules of the original duplex should result. The whole cycle could be repeated, there being added every time a fresh dose of the enzyme. It is, however, possible that upon cooling after the denaturation of the DNA duplex, renaturation to form the original duplex would predominate over the template-primer complex formation. If this tendency could not be circumvented by adjusting the concentration of the primers, clearly one would have to resort to the separation of the strands and then carry out repair replication. After every cycle of repair replication, the process of strand separation would have to be repeated".

Novelty of Claim 1 over (D4)

7. Claim 1 requires that the sequence to be amplified has to be contained within a larger sequence. Document (D4) does not relate to amplification of a sequence contained in a larger sequence, so the subject-matter of claim 1 is novel over document (D4).

Novelty of Claim 2 over (D4)

8. Claim 2 relates to a process for exponential amplification which requires at least five cycles of amplification (see section II supra).

9. In order to be novelty-destroying, a chemical compound or a process disclosed in a prior art document must have been made available to the public not as a mere chemical formula or hypothetical/theoretical process, but as a reproducible technical teaching (see eg decisions T 206/83 OJ 1987, 5 and T 902/94 of 6 March 1998). This requirement is not fulfilled by the very general suggestions of document (D4). From what is reported in Document (D4), success for even a single cycle of amplification remains uncertain, and the conditions, if any exist, for success with at least five cycles are not given. Novelty must be acknowledged for the subject-matter of claim 2 over document (D4).

Document (D9)

10. This published document emanates from the same group under Professor Khorana as document (D4). The reader is told that the group are now embarking on a different approach to that suggested in (D4), but there is a reference at page 5220, r-h column, first paragraph of document (D9) to unpublished work performed by a member

of the group, Dr Molineux pertaining to the replication of relatively short DNA duplexes by means of a "repair replication" process based on the hypothetical process referred to in document (D4). It was stated that these experiments showed that "[(b)] to form the appropriate primer-templates complexes, it was necessary to heat and cool in the presence of an excess (10 times or more) of the appropriate primers and [(c)] in order to perform multiple cycles of repair replication, it was necessary to add, after each cycle, fresh amounts of the primers so as to maintain the appropriate primer-template ratios". No details of what was actually done are given, nor is the reader told how many cyles of repair replication were achieved.

Novelty of Claim 1 over (D9)

11. Claim 1 requires that the sequence to be amplified has to be contained within a larger sequence. Document (D9) does not relate to amplification of a sequence contained in a larger sequence, so the subject-matter of claim 1 is novel over document (D4).

Novelty of Claim 2 over (D9)

12. Claim 2 relates to a process for exponential amplification which requires at least five cycles of amplification (see section II supra). Document (D9) does not even report that such number of cycles was achieved, let alone give a reproducible example of this. Novelty must be acknowledged for the subject-matter of claim 2 over document (D9).

Documents (D3) and (D7)

13. The appellants argue that NIH Grant Application (D3) and Research Proposal (D7), also relating to Professor Khorana's group were publicly available. There is, however, no evidence before the board that these documents were actually available to the public, as required of the purposes of Article 54(2) EPC. In the absence of such evidence and given that the purpose of a Grant Application or a Research Proposal being to obtain funding, not to disclose anything to the public (see decision T 1212/97, supra), the Board cannot treat these as forming part of the prior art.

14. But even if documents (D3) and (D7) were part of the prior art, neither, in the board's judgement, adds anything to the content of document (D4) in terms of "repair replication" since they merely relate to the same "hypothetical" process as disclosed in document (D4), and thus like that document do not take away the novelty of claim 1 or claim 2 for the reasons stated above in relation to document (D4). The drawings on page 37 (document (D3)) or page 18 (document (D7)) illustrate indeed the same principle set out in document (D4), according to which the "The DNA duplex would be denatured to form single strands. This denaturation step would be carried out in the presence of a sufficiently large excess of the two appropriate primers. Upon cooling, one would hope to obtain two structures, each containing the full length of the template strand appropriately complexed with the primer. DNA polymerase will be added to complete the process of repair replication. Two molecules of the original duplex should result. The whole cycle could be repeated,

there being added every time a fresh dose of the enzyme".

15. No case for lack of novelty of claims 1 and 2 thus exists on the basis of documents (D3) and (D7).

Oral presentation by Dr K. Kleppe: document (D48)

16. Document (D48) is the manuscript of a lecture given on 18 June 1969 by Dr K. Kleppe, a member of Professor Khorana's group at a Gordon Research Conference. Its contents cannot be regarded as forming part of the prior prior art already in view of the restrictions imposed on persons attending such a Gordon Research conference (cf. decision T 838/97 of 14 November 2000). Nor does the board have any satisfactory evidence what actually would have been conveyed to a member of the audience on this occasion.

17. For the sake of completeness however, and because of its possible relevance to other issues, the actual contents of the manuscript of the lecture (document (D48)) will be considered. Figure 10 thereof shows in schematic form the "repair replication" technique up to the first cycle, yielding two duplexes starting from one single duplex. Fig. 11 of document (D48), relating to the incorporation of radioactive ^{14}C-dCTP in the strands, illustrates a first plateau in the upper curve, corresponding to the first cycle depicted in Figure 10, yielding two (starting) "cold" strands and two (repaired) "hot" strands. The cpms for the first cycle are about 4,000 of incorporated ^{14}C-dCTP in the two "hot" strands. The upper curve then reaches a second plateau corresponding to the second cycle, supposed to

yield in total two (starting) "cold" strands and six (repaired) "hot" strands, provided exponential amplification occurs, in which case one would expect to measure 12,000 cpm of incorporated ^{14}C-dCTP (2 "hot" strands = 4,000 cpm; 6 "hot" strands = 12,000 cpm). Yet, the second plateau in Figure 11 is actually at 8,000 cpm, indicating that only two new "hot" strands form during the second cycle, instead of the four expected in an exponential amplification process. The fact that apparently **only** two new "hot" strands (4,000 cpms) form at each cycle would be consistent with **linear** amplification of only the original strands, or there might be other explanations. However the experiment does not show that in the second cycle each of strands, either old or newly formed in the first cycle, serves as a template which extended by repair replication in the second cycle. This is the exponential increase required by both claim 1 and claim 2, and so these claims are novel even if the contents of the lecture were made publicly available.

Document (D6)

18. Document (D6) is another document concerned with repair reactions. At page 211, last paragraph of document (D6) it suggests the addition of one single primer ("The duplex could be subjected to a repair reaction by the DNA polymerase of Escherichia coli, the repaired strands separated and the separated strands could again be annealed with **a partly complementary polydeoxynucleotide** and the repair reaction could be repeated" (emphasis added by the board). The reader is not given the details of any working example, or told what conditions should be used.

19. Both claim 1 and claim 2 require there to be two primers (see step (a) of each of these claims). Novelty of both claims thus has to be acknowledged over document (D6). A further ground for acknowledging novelty is that the document does not contain any teaching reproducible as such, but would require the reader to research for himself the conditions, if any exist, required for success.

20. Since claims 3 to 19 all refer back to the novel process of claims 1 or 2, these claims are also novel. In conclusion no novelty attack has been made out against any of the claims before the board.

Inventive step
Starting point in the prior art and problem to be solved

21. Both claims 1 and 2 are concerned with amplifying double-stranded nucleic acid sequences. According to the prior art this was already successfully done by

cloning, as suggested for example in document (D21)(see page 6614, l-h column).

22. According to the appellants the prior to be taken as the starting point is a document concerned with repair replication, such as document (D4) emanating from Professor Khorana's group. However, as discussed above in connection with novelty, this group had itself abandoned this line of research some ten years before the priority date of the present patent, and had not published any results of successful experiments with sufficient detail for others to reproduce for themselves. While the work of Professor Khorana's group may have already suggested many of the features now claimed, the skilled person at the priority date, not having hindsight, would have started from the known successful way of amplifying double-stranded nucleic acid sequences as closest prior art.

23. The problem to be solved over this closest prior art of amplification by cloning can be stated to be finding an alternative method of amplifying specific double-stranded nucleic acid sequences. In view of the considerable detail given in the patent in suit of ways to carry this out successfully, this problem can be regarded as solved by the methods of each of claim 1 and claim 2.

24. To solve this problem the skilled person would have considered *inter alia* the repair replication documents, in particular document (D4). However the skilled person would only then seriously contemplate this method, if convinced that it can be applied using only routine methods and without having to do any further research

of his own. Document (D4) does not show that success has been achieved. The skilled person would thus have first looked at the other available literature on the "repair replication" approach. He would come across document (D9) from the same team but published four years later, and see that from page 5213, they had given up on "repair replication" and adopted a different approach. The skilled person would find nothing that would make him consider that development of the "repair replication" route had any reasonable expectation of success. Even if all the "repair replication" documents were considered together and it could be assumed that they had all had been made available to the public, the skilled reader would still gain no confidence that the method could be got to work, without further research whose outcome remained uncertain, given that the very group which had suggested this line of research appeared from the literature to be abandoning it. Whereas nowadays PCR may seem routine, at the time of filing the present application this line of research was treated as leading nowhere.

25. Finally, it is remarkable that document (D4), the earliest document dealing with an hypothetical process ("repair replication") resembling exponential amplification is dated as far back as 1971, and a number of years elapsed between the publication of document (D4) and the earliest priority date of the patent in suit (1985). In the board's opinion, giving new life to a long-abandoned line of research is a further indicator of inventive step (see eg decision T 330/92 of 10 February 1994), in the sense that the elements underlying the exponential amplification of

present claims 1 or 2, such as the templates, the primers, the polymerases and the knowledge about hybridization and separation of DNA strands had long been there, but those skilled in the field have nevertheless remained "blind", the method of choice remaining amplification by in vivo cloning until the priority date of the patent in suit (see eg document (D78), point 12).

Oligonucleotides as only bottleneck?

26. The appellants sought to argue that the group headed by Prof. Khorana did not attempt to carry out a substantial number of cycles of "repair replication" because this would have required a prohibitively high quantity of oligonucleotide primers, which were scarce.

27. However, the board observes that the experiments performed by Dr K. Kleppe involve the use of 50 to 100 µl buffer containing 2 nmol/ml of primer (see page 346 of document (D4), end of first paragraph, in combination with the Legend to Figure 10). Likewise, the exponential amplification technique disclosed on page 13 of the patent in suit involves 100 µl of buffer (line 55) containing 100 pmol (line 56) of "primer A", ie at a concentration of 100 pmol/100 µl = 1 nmol/ml (similar values can be seen also on page 14, lines 30 to 31 and 50 to 52 and page 16, lines 9 to 10 of the patent in suit). Therefore, the conclusion cannot be drawn that Dr K. Kleppe had insufficient amounts of oligonucleotide primers to perform the exponential amplification of DNA.

28. Nor did Dr Kleppe have insufficient amounts of template, as he could use 100 to 500-fold or greater concentrations of template compared with the Examples of the patent in suit. The group headed by Prof. Khorana could thus have been in a position to put into practice exponential amplification with the quantities of primers and templates they had at hand. The only missing element, in the board's view, was the "forma mentis" to do so, as the authors of document (D4) had nothing else in mind than increasing the amount of a given tRNA gene and doubts arise whether they ever had any clue to the exponential nature of what they called "repair replication" and to its amazing power of amplification of trace amounts of nucleic acids. In fact, the term "exponential" never turns up in any of the "repair replication" papers. The board is not in a position to say why the earlier research apparently failed, other than that nobody believed it would work well in practice.

29. In view of the foregoing, it must be concluded that the subject-matter of both claims 1 and 2 satisfies the requirement of Article 56 EPC. Claims 3 to 19 all depend on the inventive process of claims 1 or 2, and thus are inventive as well.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar: The Chairwoman:

P. Cremona U. M. Kinkeldey