DECISION of 20 October 2003

Case Number: T 0216/96 - 3.3.4
Application Number: 86302298.4
Publication Number: 0200362
IPC: C12Q 1/68
Language of the proceedings: EN

Title of invention:
Process for amplifying, detecting, and/or cloning nucleic acid sequences

Patentee:
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Opponents:
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APPLIGENE S.A.
CIS Bio International
Rhône Poulenc Agriculture Limited
Abbott Laboratories
Pasteur Sanofi Diagnostics

Intervener:
Organon Teknika N.V.

Headword:
PCR/HOFFMANN-LA ROCHE AG

Relevant legal provisions:
EPC Art. 123(2), 83, 54, 56, 88(4)
Keyword:
"Added subject-matter (no)"
"Sufficiency of disclosure (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
G 0002/98, G 0001/93, T 0009/81, T 0305/87, T 0838/97,
T 0330/92

Catchword:
-
Case Number: T 0216/96 - 3.3.4

DECISION
of the Technical Board of Appeal 3.3.4
of 20 October 2003

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<td>Decision under appeal:</td>
<td>Decision of the Opposition Division of the European Patent Office posted 15 December 1995 rejecting the opposition filed against European patent No. 0200362 pursuant to Article 102(2) EPC.</td>
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Composition of the Board:

Chairwoman: U. M. Kinkeldy
Members: R. E. Gramaglia
S. C. Perryman
Summary of Facts and Submissions

I. European Patent No. 0 200 362 (application No. 86 302 298.4) claiming priorities from US 716975 of 28 March 1985 (P1), US 791308 of 25 October 1985 (P2) and US 828144 of 7 February 1986 (P3) was filed on 27 March 1986. The patent relates to a process for amplifying and detecting nucleic acid sequences which has now become known as the polymerase chain reaction (PCR) and was granted on the basis of 13 claims, of which independent claims 1, 8, 9 and 13 read as follows:

"1. A process for detecting the presence or absence of at least one double-stranded nucleic acid sequence in a sample, or distinguishing between two different double-stranded nucleic acid sequences in said sample, which process comprises first exponentially amplifying the specific sequence or sequences (if present) by the following steps, and then detecting the thus-amplified sequence or sequences (if present):

(a) separating the nucleic acid strands in the sample and treating the sample with a molar excess of a pair of oligonucleotide primers for each different specific sequence being detected, one primer for each strand, under hybridizing conditions and in the presence of an inducing agent for polymerization and the different nucleoside triphosphates such that for each of said strands an extension product of the respective primer is synthesized which is substantially complementary to the strand, wherein said primers are selected so that each is complementary to one end of the sequence to be amplified on one of the strands such that the extension product synthesized from one primer, when it is..."
separated from its complement, can serve as a template for synthesis of an extension product of the other primer of the pair;

(b) treating the sample resulting from (a) under denaturing conditions to separate the primer extension products from their templates;

(c) treating as in (a) the sample resulting from (b) with oligonucleotide primers 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 exponential amplification of the nucleic acid sequence or sequences, if present, results thus permitting detection thereof; and, if desired,

(e) adding to the product of step (c) or (d) a labelled oligonucleotide probe capable of hybridizing to said sequence being detected; and

(f) determining whether said hybridization has occurred.

8. A process for cloning into a vector a specific double-stranded nucleic acid sequence or sequences contained in a nucleic acid or a mixture of nucleic acids, which process comprises first amplifying said sequence or sequences, by the following steps:
(a) separating the nucleic acid strands and treating the nucleic acid(s) with a molar excess of a pair of oligonucleotide primers for each different specific sequence being amplified and cloned, one for each strand, under hybridizing conditions and in the presence of an inducing agent for polymerization and the different nucleoside triphosphates such that for each of said strands an extension product of each primer is synthesized which is complementary to its respective 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 the extension product synthesized from one primer, when it is separated from its complement, can serve as a template for synthesis of the extension product of the other primer of the pair and wherein said primers each contain a restriction site which is the same as or different from the restriction site(s) on the other primer(s);

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

(c) treating as in (a) the single-stranded molecules generated from step (b) with oligonucleotide primers 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 step (b) and (c) at least once;

whereby exponential amplification of the nucleic acid sequence or sequences results;
(e) adding to the product of step (c) or (d) a restriction enzyme for each of said restriction sites to obtain cleaved products in a restriction digest; and

(f) ligating the cleaved product(s) into one or more cloning vectors.

9. A process for synthesizing a nucleic acid fragment from an existing double-stranded nucleic acid sequence having fewer nucleotides than the fragment being synthesized and two oligonucleotide primers, wherein the nucleic acid being synthesized is comprised of a left segment, a core segment and a right segment, and wherein the core segment represents at least substantially the nucleotide sequence of said existing nucleic acid sequence, and the right and left segments represent the nucleotide sequence present at one end of each of the two primers, the other ends of which are complementary or substantially complementary to ends of the single strands produced by separating the strands of said existing nucleic acid sequence, which process comprises:

(a) treating the strands of said existing fragment 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 nucleoside triphosphates such that for each strand an extension product of the respective primer is synthesized which is complementary to the strand, wherein said primers are selected so as each to be substantially complementary to one end of a strand of said existing sequence such that the extension
product synthesized from one primer, when it is separated from its complement, can serve as a template for synthesis of an extension product of the other primer, and wherein each primer also contains a sequence of nucleotides which are not complementary to said existing sequence and which correspond to the two ends of the nucleic acid fragment being synthesized;

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

(c) treating the single-stranded molecules generated from step (b) with the primers of step (a) under conditions such that a primer extension product is synthesized using each of the single strands produced in step (b) as a template so as to produce two intermediate double-stranded nucleic acid molecules, into each of which has been incorporated the nucleotide sequence present at one end of the oligonucleotide primers, and two full-length double-stranded nucleic acid molecules, into each of which has been incorporated the nucleotide sequence present at one end of both of the oligonucleotide primers;

(d) repeating steps (b) and (c) for a sufficient number of times to produce the full-length double-stranded molecules in an effective amount.

13. A kit for the detection of at least one specific nucleic acid sequence in a sample, which kit comprises, in packaged form, a multicontainer unit having:
(a) each of two oligonucleotide primers for each
different sequence to be detected, wherein

(i) if the specific nucleic acid sequence to be
detected is single-stranded one primer is substantially
complementary to one end of the strand so that an
extension product of said one primer formed under
hybridizing conditions and in the presence of an
inducing agent for polymerization and the different
nucleoside triphosphates is substantially complementary
to said strand, and the other primer is substantially
complementary to one end of said extension product and
can be used under hybridizing conditions and in the
presence of an inducing agent for polymerization and
the different nucleoside triphosphates to synthesize
another extension product employing said extension
product of said one primer as a template thereby
providing a nucleic acid of two strands; or

(ii) if the specific nucleic acid sequence to be
detected is double-stranded the primers are such that
each is substantially complementary to one end of one
of the strands and an extension product synthesized
from each primer using its complement strand as a
template under hybridizing conditions and in the
presence of an inducing agent for polymerization and
the different nucleoside triphosphates, when separated
from its complement can serve as a template for
synthesis of an extension product of the other primer
under hybridizing conditions and in the presence of an
inducing agent for polymerization and the different
nucleoside triphosphates;

(b) an agent for polymerization;
(c) each of the different nucleoside triphosphates;

(d) an oligonucleotide probe capable of hybridizing to said sequence if it is present in said sample; and

(e) means for detecting hybrids of said probe and said sequence."

Claims 2 to 7 were addressed to specific embodiments of the process according to claim 1 or to the preceding claims, whereas claims 10 to 12 related to specific embodiments of the process of claims 8 and/or 9.

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. A notice of intervention was filed by the intervener, after the opposition division had already orally announced the decision to reject the oppositions, but the intervention was subsequently withdrawn.

III. Appellants III, IV and VI (opponents (03), (04) and (06)) filed appeals against the decision of the opposition division. Appellant VI took over the arguments of the intervener and considered it as being "part of the present statement of grounds". Opponent (02) filed but later withdrew an appeal and is party to the appeals pursuant to Article 107 EPC, as is opponent (05).

IV. In a communication of 23 July 1996, the board expressed its provisional opinion about the admissibility of these appeals.
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 to 361 (1971);

(D6) Research Proposal (period from 1 February 1973 to 31 January 1978) submitted by Prof. H.G. Khorana at the National Science Foundation;

(D7) Khorana H.G. et al., J. Mol. Biol., Vol. 72, pages 209 to 217 (1972);


(D20) US-A-4,293,652;
(D25) Rossi J.J. et al., J. Biol. Chem., Vol. 16, pages 9226 to 9229 (1982);

(D32) Reyes A.A. and Wallace R.B., Genet. Eng., Vol. 6, pages 157 to 173 (1984);

(D46) Wallace R.B. et al., Biochimie, Vol. 67, pages 755 to 762 (1985);

(D62) Manuscript of a lecture given by Dr K. Klücke at the Gordon Research Conference on 18 June 1969;

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

(D84) Declaration of F.A. Faloona dated 10 February 1995;

(D84.2) Mullis K.B. et al. in "The Polymerase Chain Reaction", Birkhäuser, Boston, Basel, Berlin, pages 430 to 432;

(D84.3) Mullis K.B. Testimony in the case Hoffmann-La Roche vs Promega Corporation, pages 31 to 33, 86 to 88 and 116;


(D87) Winter G. et al., Nucleic Acid Research, Vol. 9, No. 2, pages 237 to 245 (1981);
VII. The written submissions by the appellants and the other parties are summarized as follows:

**Article 123(2) EPC**

- In claim 1 and the application, both as filed, the detection of the amplified sequence by hybridization with a labelled oligonucleotide probe was a critical feature. However, in granted claim 1 this feature had become optional (cf "if desired"). Therefore, granted claim 1 had been
extended to detection methods other than hybridization with a labelled oligonucleotide probe (steps (e) and (f)), for which there was no basis in the application as filed.

Whereas claim 8 as filed was restricted to a multi-container unit wherein each of the reagents (a) to (e) had to be in a separate container, granted claim 13 was no longer restricted to this embodiment. Therefore claim 13 extended the subject-matter as filed.

Article 83 EPC

The patent specification was not enabling for the embodiment of claim 1 wherein the starting material was double stranded RNA and wherein the amplified material was still RNA throughout the amplification process.

The kit of claim 13 could be used in other amplification methods which were insufficiently disclosed in the patent in suit, namely (i) RNA amplification via formation of new RNA strands and (ii) amplification without separation of the strands. Therefore, the subject-matter of claim 13 did not comply with the requirements of Article 83 EPC.

Novelty
Oral disclosure by Dr Mullis

According to document (D84), 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 disclosed orally the PCR concept to third parties outside the Cetus company, his employer. The facts mentioned in document (D84) were confirmed by document (D84.2) and by the Dr Mullis Testimony (document (D84.3)).

Oral disclosure by Dr Kleppe

- Claim 1 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 (D62)) showed in schematic form the DNA replication 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".

Documents (D4), (D3), (D6), (D7) and (D8)

- Claim 1 related to the combination of exponential amplification with the detection of the amplified product. The "repair replication" amplification method disclosed by documents (D4), (D3), (D6), (D7) and (D8) automatically also comprised the verification of the presence of the amplified material, e.g. by measuring the rate of radioactivity incorporation in the newly
synthesised strands, and thus also comprised all the features of claim 1.

- Since the detection steps (e) and (f) in claim 1 were optional (cf "if desired"), the claimed method was indistinguishable from the repair replication method disclosed by documents (D4), (D3), (D6), (D7) and (D8).

- Claim 8 related to an amplification procedure, wherein each primer comprised a restriction site. After amplification, restriction enzymes were added and the cleaved product ligated into a vector. The claim lacked novelty in view of the fact that restriction enzymes and techniques for introducing nucleic acid sequences into vectors were known at the priority date of the patent in suit.

- Claim 9 related to an amplification procedure, wherein the primers protruded from the template. The claim lacked novelty because it was self-evident that protruding primers still hybridized.

- Since the individual components (a) to (e) of the kit of claim 13 failed to form the functional unity through a purpose-directed application, as required by eg decision T 9/81 (OJ EPO 1983, 372), the claim recited the mere aggregation of known elements (a) to (e), independently of the process as defined in claim 1. It was thus anticipated by documents (D32), (D86), (D88), (D89), (D90) and (D94) because these documents disclosed a process involving the use of (a) two oligonucleotide
primers; (b) an agent for polymerization; (c) each of the different nucleoside triphosphates; (d) an oligonucleotide probe and (e) detecting means.

Inventive step

- On the one hand, it was already known from documents (D32), (D46) and (D19) that an oligonucleotide probe could fish out a DNA or mRNA with high specificity within a complex mixture of DNAs or RNAs. On the other hand, documents (D3), (D4), (D7) and (D8) taught how to increase the number/amount of a specific DNA with respect to the concentration of other DNAs within a mixture. Therefore claim 1, encompassing the combination of exponential amplification with the detection of or the discrimination between nucleic acids was obvious.

- The use of primers containing a restriction site in the amplification process of claim 8 was obvious in view of documents (D20) and (D25), disclosing the introduction at both ends of a double stranded DNA of linkers containing a restriction site to permit its insertion into a vector.

- Claim 9 related to a process for elongating both ends of a nucleic acid during amplification, so as to obtain a final product which was longer than the existing double stranded nucleic acid sequence. However, the claim lacked inventive step because it was known from documents (D4) and (D14) that
repair replication occurred, regardless of the primer position vis-à-vis the template,

whenever a priming effect took place in the presence of a DNA polymerase and the four different deoxyribonucleoside triphosphates.

Documents (D4) and (D62) disclosed exponential amplification of nucleic acids, while documents (D95), (D32) and (D94) disclosed oligonucleotide probes and their use for detecting a target nucleic acid. Therefore, the subject-matter of claim 13 lacked inventive step vis-à-vis the combination of documents (D4) or (D62) with any of documents (D95), (D32) and (D94).

The kit of claim 13 was obvious in the light of documents (D86), (D87), (D88), (D89) and (D90), as the mere packaging of the components (a) to (e) known from these documents in a multicomponent unit could not provide an inventive step.

As regards the wording "in packaged form a multicontainer unit" in claim 13, this feature was disclosed for the first time in priority document (P3) (see paragraph I supra). Therefore, with respect to the "packaged form/ multicontainer unit" language, priority could only be invoked on the basis of priority document (P3) of 7 February 1986. Consequently, the kit of claim 13 was obvious in view of the combination of documents (D91) and (D92) published before that date, disclosing a kit for performing exponential
amplification and a kit in a multicontainer packaged form, respectively.

VIII. The written submissions by the respondent are summarized as follows:

**Article 123(2) EPC**

- The application as filed was not restricted to the detection of the amplified product by means of a labelled oligonucleotide probe. Column 2, last five lines of the published ("A") application as filed related to a process that encompassed amplification and detection in general.

- It could be derived from the application as filed that there was no requirement that all components of the kit of claim 13 had to be in separate containers.

**Article 83 EPC**

- Since claim 1 related to a process for "detecting the presence or absence of at least one double-stranded nucleic acid sequence in a sample", there was no requirement that, departing from a double stranded RNA, the amplified product had still to be RNA.

**Novelty**

*Oral disclosure by Dr Mullis*

- As regarded any of the oral disclosures by Dr Mullis (referred to in document (D84)), it
could not be deduced from document (D64) 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 (D62) 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 (D62) 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.

Documents (D4), (D3), (D6), (D7) and (D8)

- These documents were either not publicly available (documents (D3) and (D6)) or irrelevant because they were not enabling for exponential amplification. In any case, none of the cited documents disclosed the combination of exponential amplification with the detection of the amplified product.

- No prior art disclosed the combination according to claim 8 of an amplification procedure with the
introduction of restriction sites at the extremes of the amplified product.

- No prior art disclosed the combination according to claim 9 of an amplification procedure with the simultaneous elongation of the template.

- The kit of claim 13 could be produced and used for the amplification and detection of at least one specific nucleic acid sequence in a sample. The individual elements (a) to (e) of the kit of claim 13 formed the required functional unity through a purpose-directed application since the kit contained two primers for use in amplification and a probe capable of detecting the desired target sequence in a sample. Such combination of elements with such an indication of scope could not be derived from documents (D32), (D88) and (D89).

**Inventive step**

- The method of claim 1, including exponential amplification and detection of or discrimination between nucleic acids was not obvious since the method of exponential amplification was inventive as such and the prior art did not suggest such a combination. There was also no reasonable expectation of success as to whether exponential amplification would provide sufficient enrichment of the target sequence alone to allow reliable detection.
- The method of claim 8 related to exponential amplification wherein the primers contained a restriction site in order to allow the cloning of a particular nucleic acid sequence. No prior art document suggested such an approach.

- Claim 9 related to a process wherein a nucleic acid fragment could be synthesized from an existing double stranded nucleic acid sequence having fewer nucleotides. The subject-matter of this claim involved an inventive step since none of documents (D4) and (D14) disclosed the elongation at its ends of an existing double stranded nucleic acid sequence.

- The indication of the purpose (amplification and detection of nucleic acids) for the kit of claim 13 was sufficient to establish inventive step.

- Priority document (P1) disclosed all the components of the kit of claim 13, the combination of said components and their use. Therefore, the kit of claim 13 could enjoy the filing date of document (P1) for the purpose of the right to priority and documents (D91) and (D92) did not represent prior art useful for questioning the inventive step of kit claim 13.

IX. The appellants requested that the decision under appeal be set aside and that the patent be revoked.

The respondent requested that the appeals be dismissed.
Reasons for the Decision

1. The appeals by opponents (03), (04) and (06) (appellants III, IV and VI, respectively) are admissible.

Article 123(2) EPC

2. Owing to the fact that steps (e) and (f) in claim 1 at issue are optional, the appellants argue that the claim has been extended to cover other detection means for which there was no basis in the application as filed, which disclosed steps (e) and (f) as obligatory features.

However, the board notes that there is a basis in column 2, last five lines of the published "A" application as filed for a process that encompasses amplification and detection in general. It is true that detection by hybridization with a labelled oligonucleotide probe is a preferred embodiment, however, other detection methods such as incorporation of the label during amplification (column 26, lines 46 to 54) or electrophoresis and staining of the gel (see Example 6) are also disclosed in the application as filed.

3. It is argued by the appellants that claim 13 extends the subject-matter as filed, because this claim, unlike claim 8 as filed, is not restricted to a multi-container unit wherein each of the reagents (a) to (e) has to be in a separate container.
However, according to the application as filed, there is no requirement that all components of the kit of claim 13 have to be each in a separate container. This is because column 35, lines 7 to 15 of the published application as filed illustrates the simultaneous addition of the primers and the four nucleotides triphosphates. Column 35, lines 43 to 52 (ibidem) shows the addition of "Solution A" comprising the primers and the four nucleotides triphosphates, while column 21, lines 42 to 49 (ibidem) discloses the addition of a solution comprising the agent for polymerization and the four nucleotides triphosphates. With regard to Article 123(2) EPC, the underlying idea is to safeguard that the public will not be taken by surprise by a claim reworded during examination and/or opposition/appeal proceedings to cover subject-matter which would not explicitly or implicitly be derivable by a skilled person from the whole content of the application as filed. In the present situation, the public would have looked at the whole of the technical subject-matter described in the application as filed (see decision G 1/93, O.J. EPO, 1994, 541, point 9 of the "reasons"), and not just the originally filed claims.

4. In view of the above findings, the board concludes that no case has been made out that claims 1 and 13 do not satisfy the requirements of Article 123(2) EPC.

Article 83 EPC

5. The appellants maintain that the patent is not enabling for the embodiment of claim 1, wherein the starting material is double stranded RNA and wherein the
amplified material is still RNA throughout the amplification process until detection occurs.

While it is true, in the board's judgement, that the wording in claim 1 "a process for detecting the presence or absence of at least one double-stranded nucleic acid sequence in a sample" is addressed to the detection of both DNAs and RNAs, the claim does not require as a critical technical feature that, in the case of RNA detection, the template has still to be RNA throughout the amplification process. The decisive criterion when considering the requirements of Article 83 EPC is that the patent specification enables the skilled person to perform amplification and detection of double stranded RNA as stated in claim 1. It is not disputed by the appellants that the claimed method allows detection of a double-stranded RNA via reverse transcription (see page 8, line 57: "reverse transcriptase") of the RNA strands into the corresponding cDNAs and then amplification of the cDNA strands.

6. In the appellants' view, the subject-matter of claim 13 does not satisfy the requirements of Article 83 EPC because the kit of claim 13 could be used in other amplification methods which are insufficiently disclosed in the patent in suit, namely (i) RNA amplification and detection via the formation of new RNA strands and (ii) amplification and detection without separation of the strands.

Yet it cannot be disputed that the kit of claim 13 can be produced as such and can be used for the purpose stated in claim 13, namely "for the amplification and
detection of at least one specific nucleic acid sequence in a sample". The description of the patent in suit, Example 2, part I indeed gives the necessary information for the synthesis and characterisation of oligonucleotides complementary to a specific template. Examples 4 and 8 are two of the examples describing the amplification and detection of specific templates using the claimed kit. Means for detecting the amplified sequences are also disclosed, in particular in Examples 6 and 11. The board does not agree with the appellants' contention that claim 13 must be read as also covering a kit for carrying out the "exotic" embodiments (i) and (ii) above. Thus no question can arise under Article 83 EPC of whether the kit is adequate for carrying out such embodiments.

7. In view of the foregoing, the board concludes that the requirements of Article 83 EPC are fulfilled.

Novelty (Article 54 EPC)

Oral disclosure by Dr Mullis

8. Relying on documents (D84), (D84.2) and (D84.3) 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 (D84) 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.
Document (D84.2) 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.

Document (D84.3) is part of a deposition made in US court proceedings between Hoffmann-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.

All this does not represent 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, on the basis of which the board would be convinced that anything was made available to the public by the inventor which can be used as prior art to attack the patent in suit.

Oral presentation by Dr K. Kleppe

9. The oral presentation made by Dr K. Kleppe, of which document (D62) is a manuscript, does 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 do not form prior art.
Claims 1 to 7

Documents (D3), (D4), (D6), (D7) and (D8)

10. Claim 1 relates to a process comprising (i) at least two cycles of exponential amplification of polynucleotides (see steps (a) to (c)) in combination with (ii) a step of detection of the amplified product. It is the appellants' view that present claim 1 lacks novelty vis-à-vis documents (D4), (D3), (D6), (D7) and (D8). It is argued that the "repair replication" method disclosed in these documents not only anticipates (i) at least two cycles of exponential amplification of polynucleotides but also automatically comprises (ii) the verification of the presence of the amplified material (eg by measuring the radioactivity incorporation in the newly-synthesized strands). Therefore, the "repair replication" method disclosed in documents (D4), (D3), (D6), (D7) and (D8) comprises all the features of claim 1 at issue.

11. It is noted that documents (D4), (D3), (D6), (D7) and (D8) all originate from a team headed by Prof. Khorana at the Institute for Enzyme Research of the University of Wisconsin, Madison. 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".

12. The respondent denies that documents (D3) and (D6) were publicly available. But even if they were, in the board's judgement, neither 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), according to
which the "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 polynucleotide and the repair reaction could be repeated". Nor do documents (D7) and (D8) go beyond document (D4) regarding the above mentioned hypothetical process.

13. Even assuming that documents (D4), (D3), (D6), (D7) and (D8) were prior art according to Article 54(2) EPC and that they disclosed (i) at least two cycles of exponential amplification of polynucleotides, in the board's judgement, they fail to disclose the combination of (i) with (ii), the latter being the detection of the amplified product.

14. The appellants maintain that the prior art cited above automatically also comprises the detection/verification of the presence of the amplified material, eg by measuring the rate of radioactivity incorporation in the newly synthesised strands, and thus also comprises all the features of claim 1, the more so as the detection steps (e) and (f) in claim 1 are optional (cf "if desired").

15. The board accepts that the documents cited by the appellants disclose measuring the incorporation of eg [\(\alpha^{32}\text{P}\)]-labelled deoxynucleotide triphosphates in order to follow the completion of the repair reaction (see eg document (D4), legend to Figure 5 on page 347). However, this prior art is concerned with repair of a DNA sequence known to be present, not with detection of whether or not a DNA sequence is present. Detection of
repair is a separate concept and does not mean that the process of claim 1 is carried out.

16. Secondly, the authors of these documents were merely looking for a method for increasing the amount of their synthetic, complete tRNA genes. The prior art thus does not disclose the feature (ii) of claim 1 relating to the detection of the presence or absence of a specific polynucleotide in a sample or to distinguishing between two different polynucleotides in a sample, a problem which did not arise at all. Hence, the concept underlying present claim 1 of "finding the needle (possibly in a haystack) by amplifying the needle" cannot at all be derived from these documents.

17. The appellants also maintain that the detection steps (e) and (f) in claim 1 are recited as optional features (cf "if desired") and that therefore the claimed method is indistinguishable from the repair replication method disclosed by the prior art.

The board accepts that, because of the wording "if desired" in claim 1 at issue, steps (e) and (f) relating to the detection via a labelled oligonucleotide, are optional. However, it is this specific detection method only which is optional. In fact, the preamble of claim 1 ("A process for detecting the presence or absence of at least one double-stranded nucleic acid sequence in a sample, or distinguishing between two different double-stranded nucleic acid sequences in said sample, which process comprises first exponentially amplifying the specific sequence or sequences (if present) by the following steps, and then detecting the thus-amplified sequence or sequences")
(emphasis added by the board)) makes an (unspecified) detection step mandatory.

18. In view of the above findings, the board concludes that the process of claim 1 and thus dependent claims 2 to 7 is in accordance with the requirements of Article 54 EPC.

Claims 8 to 12

19. No prior art discloses the combination according to independent claim 8 of at least two cycles of exponential amplification of polynucleotides (see steps (a) to (c)) with the introduction of restriction sites at the extremities of the amplified product, followed by the addition of restriction enzymes and ligation of the cleaved product into a vector. Nor does any prior art disclose the combination according to independent claim 9 of at least two cycles of exponential amplification of polynucleotides with the simultaneous elongation of the template by means of protruding primers. These claims and dependent claims 10 to 12 thus satisfy the requirements of Article 54 EPC.

Claim 13

20. The appellants' line of argument for questioning the novelty of the kit of claim 13 is based on the contention that the individual components (a) to (e) of the kit do not achieve a functional unity through a purpose-directed application, as required by eg decision T 9/81 (OJ EPO 1983, 372). Hence, the appellants view claim 13 as covering a mere aggregation of known elements (a) to (e), independently of the
process as defined in claim 1, which aggregation of elements is anticipated by documents (D32), (D86), (D88), (D89), (D90) and (D94) disclosing a process involving the use of (a) two oligonucleotide primers; (b) an agent for polymerization; (c) each of the different nucleoside triphosphates; (d) an oligonucleotide probe and (e) detecting means.

21. However, the board observes that the preamble in claim 13 "a kit for the detection of at least one specific nucleic acid sequence in a sample" and the following wordings in the claim: "two oligonucleotide primers for each different sequence to be detected", "the primers are such that each is substantially complementary to one end of one of the strands" and "a labelled oligonucleotide probe capable of hybridizing to said sequence if it is present in said sample" necessarily implies that the kit is being used in a process according to claim 1 for detecting a nucleotide sequence looked for that has been previously amplified. These expressions also unambiguously define which interactions the kit’s components have to exert upon each other. Therefore, the conclusion cannot be drawn that a kit comprising items (a) to (e) as defined above can also be used in processes other than that of claim 1. In conclusion, the kit of claim 13 achieves a functional amalgamation through a purpose-directed application, as required by eg decision T 9/81 (supra).

22. The decisive question is thus whether or not documents (D32), (D86), (D88), (D89), (D90) and (D94) disclose the combination of elements (a) to (e) and the scope aimed at stated in claim 13, bearing in mind that the content of a prior art document cannot be treated as a
reservoir from which it would be permissible to draw features in order to create artificially a particular embodiment which would destroy novelty, unless the document itself suggests such a combination of features (decision T 305/87, OJ EPO 1991, 429).

Document (D32)

23. Document (D32) discloses the use of labelled oligonucleotide probes for isolating and detecting cloned DNAs and also methods for synthesizing cDNA from mRNA. Reference is made on page 165 thereof (see last paragraph), to two mixtures of oligonucleotides. However, even if one were to consider these two mixtures of degenerated oligonucleotide probes as primers, such primers hybridize with two different regions of one strand encoding the factor IX protein (see ibidem). Therefore, this document does not disclose component (a) of the claimed kit as it is defined in claim 13 ("two oligonucleotide primers, wherein one primer is substantially complementary to one end of the strand and the other primer is substantially complementary to the end of either the other strand or of the extension product"), let alone the specific combination of elements (a) to (e) of claim 13.

Documents (D87) and (D88)

24. These documents fail to disclose component (d) of the claimed kit ("a labelled oligonucleotide probe capable of hybridizing to said sequence if it is present in said sample"), much less the specific combination of elements (a) to (e) of claim 13.
Document (D89)

25. The document does not disclose component (a) of the claimed kit ("two oligonucleotide primers"). Even by considering a mixture of 24 degenerated oligonucleotide probes (see Figure 1 on page 4062) as a primer, the technique of document (D89) uses only one primer complementary with one strand. In any case, the specific combination of elements (a) to (e) of claim 13 cannot be derived from this document.

Document (D90)

26. This document also fails to disclose component (a) of the claimed kit ("each of two oligonucleotide primers"). This is because the technique disclosed by document (D90) uses only one primer (see page 288, right-hand column, last paragraph). Further the specific combination of elements (a) to (e) of claim 13 cannot be derived from this document.

Document (D94)

27. The method disclosed in document (D94) requires that the extension product (cDNA strand) be treated with terminal transferase and dC to introduce an oligo(dC)-tail in order to generate a new region that is capable of hybridizing to the "second" oligo(dG)-primer (see page 222, points 4 and 5 and Figure 7.3.C on page 223). Therefore, since the "second" primer is not complementary to the end of the extension product, this document fails to disclose component (a) of the claimed kit as it is defined in claim 13 ("two oligonucleotide
primers, wherein one primer is substantially complementary to one end of the strand and the other primer is substantially complementary to the end of either the other strand or of the extension product"), not to speak about the specific combination of elements (a) to (e) of claim 13.

28. In conclusion, each of documents (D32), (D86), (D88), (D89), (D90) and (D94) refers only to some but not all of the components (a) to (e) of kit claim 13. Moreover, these "scattered ingredients" are never disclosed as a specific combination, contrary to the requirements set out in eg decision T 9/81 (supra). Therefore, the board is convinced that the kit of claim 13 satisfies the requirements of Article 54 EPC.

Inventive step (Article 56 EPC)

Claims 1 to 7

29. Claim 1 at issue being concerned with detection of nucleic acid(s) in a sample, in the board's judgement, the "repair replication" documents disclosing no such detection (see points 15 to 17 supra) cannot represent the closest prior art. The latter is rather represented by the "detection" documents (D32), (D46) and (D19) relating to the same technical problem as present claim 1.

30. The objective technical problem to be solved by the subject-matter of claim 1 at issue can be stated as the provision of a process for detecting the presence or absence of at least one double-stranded nucleic acid sequence in a sample, or distinguishing between two different double-stranded nucleic acid sequences in
said sample. The solution is the process of claim 1. On the basis of the numerous examples which are given in the patent specification, the board is satisfied that the above problem is solved by the claimed process, which comprises (i) at least two cycles of exponential amplification followed by (ii) a step of detection of the amplified product (see point 8 supra). These examples, besides giving all the necessary technical information for achieving exponential amplification of DNAs, disclose means and processes for detecting the amplified DNAs.

31. The appellants maintain that claim 1, encompassing the combination of exponential amplification with the detection of the amplified nucleic acid is obvious in the light of documents (D3), (D4), (D7) and (D8), disclosing amplification (the "repair replication" documents), combined with documents (D32), (D46) and (D19) (the "detection" documents), showing that an oligonucleotide probe can detect a DNA or mRNA with high specificity within a complex mixture of DNAs or RNAs.

32. Regardless of the starting point, the appellants' assumption that a skilled person would have combined the teaching of the "detection" documents with that of the "repair replication" ones (or vice-versa) is, in the board's judgement, the result of an ex-post-facto analysis based on the knowledge only derivable from present claim 1.

33. Departing from the "detection" documents, the skilled person faced with the problem of an improved method of detection could not derive any solution by merely
looking at the "repair replication" documents. These disclosed no method of amplification that had been successful in any circumstances, let alone under conditions where detection of a sequence whose presence was not even certain was the prime aim. The skilled person would first have to hit on the notion that amplification was the solution to the problem of providing improved detection, and then to embark on research on what to do, for which the "repair replication" documents could not inspire him/her with any expectation of success.

34. In conclusion, the method of claim 1, including exponential amplification and detection of or discrimination between nucleic acids is not obvious since it cannot be derived from the prior art in any obvious manner. Claims 2 to 7 also fulfil the requirements of Article 56 EPC, since they either directly or indirectly refer back to the method of claim 1.

Claims 8 to 12

35. Claim 8 relates to cloning into a vector a specific double-stranded nucleic acid sequence or sequences which comprises first amplifying exponentially said sequence or sequences with primers containing restriction sites, adding restriction enzymes for each of said restriction sites to obtain cleaved products in a restriction digest; and ligating the cleaved product(s) into one or more cloning vectors. According to the acknowledged prior art (eg, document (D19), page 6614, left-hand column), if numerous such vectors were to be made, this was done by first cutting the DNA.
using restriction enzymes to give the desired specific double-stranded nucleic sequence, and then ligating this into a vector, transforming a microorganism with the vector, multiplying the microorganism, and then breaking up the microorganism to release the vector containing the DNA, claim 8 differs from this prior art method in that before amplifying in a microorganism, it uses a primer/template/extension product system to amplify up DNA including the desired sequence, the primers used also having suitable restriction sites, so that the two complementary strands of the desired sequence can be obtained by cleaving by restriction enzymes.

36. The problem to be solved over this closest prior art can be stated to be finding an alternative to the known method of cloning specific double-stranded nucleic acid sequences where these sequences have been obtained by amplification by cloning in a microorganism. This problem can be regarded as solved by the subject matter of claim 8.

The skilled person when looking for an alternative to the known method, might have come across the "repair replication" of 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 available literature on the "repair replication" approach. He/she would come across document (D9) from the same team but published four years later, and see that from page 5213, that
they had given up on "repair replication" and adopted a different approach. The skilled person would find nothing that would make him/her consider that development of the "repair replication" route had any reasonable expectation of success, let alone consider how the primers should be selected relative to restriction sites. This same comment also applies to the other documents relating to "repair replication" (documents (D3), (D6) and (D7)). Even if assuming that they were all 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.

37. Moreover, in the board's opinion, giving new life to a long-abandoned line of research is already an indicator of inventive step (see eg decision T 330/92 of 10 February 1994), in the sense that the elements underlying the exponential amplification step in present claim 8, 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).

38. For these reasons the board concludes that the subject matter of claim 8 cannot be derived in an obvious manner from the prior art, and accordingly it meets the requirements of Article 56 EPC.
39. Claim 9 relates to a process of synthesizing a nucleic acid fragment from an existing double-stranded nucleic acid sequence having fewer nucleotides than the fragment being synthesized. According to the acknowledged prior art, (see e.g., document (D14), page 898, Figure 32-8, "primed single strand (linear)"), a double-stranded DNA having a protruding 5'-end is "repair replicated" to yield a double stranded DNA having more nucleotides. Claim 9 differs from this prior art method in that it uses a primer/template/extension product system to amplify up DNA including the desired sequence, the primers being protruding so as to extend the original nucleic acid fragment.

40. The problem to be solved over this closest prior art can be stated to be finding an alternative to the known method of obtaining a double stranded DNA having more nucleotides from a shorter double-stranded DNA. This problem can be regarded as solved by the subject matter of claim 9.

The skilled person when looking for an alternative to this known method, might have come across the "repair replication" of document (D4). However for the reasons set out in points 36 and 37 above he/she would not have gained any confidence that "repair replication" could be got to work, without further research whose outcome remained uncertain. Nor would the skilled reader have obtained any hint how to use the primers to simultaneously extend the DNA and exponentially amplify it, only with hindsight could this concept be deduced from the "repair replication" documents.
41. For these reasons the board concludes that the subject matter of claim 9 cannot be derived in an obvious manner from the prior art, and accordingly it meets the requirements of Article 56 EPC. This conclusion also applies to dependent claims 10 to 12

Claim 13

42. The fact that the kit of claim 13 is specifically designed for carrying out the process of claim 1 (see point 22 supra), which has been held to involve an inventive step, is sufficient to establish inventive step of the kit.

43. It is argued by the appellants that the kit of claim 13 is obvious in the light of documents (D86), (D87), (D88), (D89) and (D90), as the mere packaging of the components (a) to (e) known from these documents in a multicomponent unit cannot provide an inventive step.

The board disagrees with this contention in view of the conclusion arrived at by the board under point 22 supra that the kit of claim 13 achieves a functional amalgamation through a purpose-directed application.

44. It is the appellants' view that the kit of claim 13 lacks inventive step for the same reasons as does claim 1.

Yet, this contention has to be dismissed on the same grounds detailed under point 34 supra, according to which the skilled person would not have combined the teaching of the "repair replication" documents with that of the "detection" ones (or vice-versa).
45. Finally, the appellants maintain that owing to the "packaged form/multicontainer unit" language, claim 13 can only enjoy the filing date of document (P3) (7 February 1986) for the purpose of the right to priority and that, consequently, the kit of claim 13 is obvious in view of the combination of documents (D91) and (D92) published before that date, disclosing a kit for performing exponential amplification and a kit in a multicontainer packaged form, respectively.

All the components of the kit of claim 13, the combination of said components and their use are already referred to not only in Examples 1 to 5 of priority document (P1), but also in claim 27 thereof, merely differing from claim 13 at issue by the omission of the "packaged form/multicontainer unit" language. As regards the "multicontainer" feature, the method of eg Example 1.III (page 25) of priority document (P1) is performed by means of a multicontainer kit. As for the "packaged form" feature, this feature is implicit in the priority texts since the skilled person would understand this feature as the standard form of a kit even without any explicit mention. Therefore, the conclusion cannot be drawn that there is no identity of invention between the kit of present claim 13 and the one derivable from priority document (P1) taken in its entirety, as required by Article 88(4) EPC (see also decision G 2/98, OJ 2001, 413). Claim 13 can thus enjoy the filing date of document (P1) for the purpose of the right to priority and documents (D91) and (D92) do not represent prior art useful for questioning the inventive step.
46. In view of the foregoing, it must be concluded that the subject-matter of claims 1 to 13 cannot be derived in an obvious manner from the prior art.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar:

P. Cremona

The Chairwoman:

U. M. Kinkeldey