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D E C I S I O N
of 24 May 2005

Case Number: T 0445/04 - 3.3.04

Application Number: 91917050.6

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Title of invention:
Homogeneous assay system

Patentee:
F. HOFFMANN-LA ROCHE AG

Opponent:
The Secretary of State for Defence in her Britannic Majesty's
Government of United Kingdom and Northern Ireland

Headword:
Homogeneous assay system/F. HOFFMANN-LA ROCHE AG

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

Keyword:
"Main request: added subject-matter (no)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
-

Catchword:
-



Case Number: T 0445/04 - 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 24 May 2005

Appellant I: F. HOFFMANN-LA ROCHE AG
(Proprietor of the patent) Postfach 3255
CH-4002 Basel (CH)

Representative: Jaenichen, Hans-Rainer, Dr
VOSSIUS & PARTNER
Siebertstrasse 4
D-81675 München (DE)

Appellant II: The Secretary of State for Defence in her
(Opponent) Britannic Majesty's Government of the
United Kingdom and Northern Ireland
DSTL, Porton Down
Salisbury
Wiltshire SP4 OJQ (GB)

Representative: Greaves, Carol Pauline
Greaves Brewster LLP
Indigo House
Cheddar Business Park
Wedmore Road
Cheddar
Somerset BS27 3EB (GB)

Decision under appeal: Interlocutory decision of the Opposition
Division of the European Patent Office posted
19 February 2004 concerning maintenance of
European patent No. 0543942 in amended form.

Composition of the Board:

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

Summary of Facts and Submissions

- I. European Patent No. 0 543 942 (application No. 91 917 050.6, published as WO 92/02638) relating to a homogeneous assay system was granted on the basis of 63 claims.
- II. Notice of opposition was filed by the opponent requesting the revocation of the European patent on the grounds of Articles 100(a) and (c) EPC. The opposition division maintained the patent on the basis of the claims of the "Auxiliary Request 4" then on file.
- III. Appellant I (patentee) and appellant II (opponent) filed appeals against the decision of the opposition division.
- IV. Oral proceedings were held on 24 May 2005, during which appellant I filed a new main request (claims 1 to 55) with claims 1 and 13 being the same as granted. This request had four independent claims 1, 13, 40 and 50 reading as follows:
- "1. A process for the detection of a target nucleic acid sequence in a sample, said process comprising:
- a) contacting a sample comprising single-stranded nucleic acids with an oligonucleotide containing a sequence complementary to a region of the target nucleic acid and a labeled oligonucleotide containing a sequence complementary to a second region of the same target nucleic acid sequence strand, but not including the nucleic acid sequence defined by the first oligonucleotide, to create a mixture of duplexes during hybridization conditions, wherein the duplexes comprise

the target nucleic acid annealed to the first oligonucleotide and to the labeled oligonucleotide such that the 3' end of the first oligonucleotide is adjacent to the 5' end of the labeled oligonucleotide;

b) maintaining the mixture of step (a) with a template-dependent nucleic acid polymerase having a 5' to 3' nuclease activity under conditions sufficient to permit the 5' to 3' nuclease activity of the polymerase to cleave the annealed, labeled oligonucleotide and release labeled fragments; and

c) detecting and/or measuring the signal generated by the hydrolysis of the labeled oligonucleotide."

"13. A polymerase chain reaction (PCR) amplification process for detecting a target nucleic acid sequence in a sample, said process comprising:

(a) providing to a PCR assay containing said sample, at least one labeled oligonucleotide containing a sequence complementary to a region of the target nucleic acid, wherein said labeled oligonucleotide anneals within the target nucleic acid sequence bounded by the oligonucleotide primers of step (b);

(b) providing a set of oligonucleotide primers, wherein a first primer contains a sequence complementary to a region in one strand of the target nucleic acid sequence and primes the synthesis of an extension product and a second primer contains a sequence complementary to a region in a second strand of the target nucleic acid sequence or complementary to a region in the extension product of the first primer and primes the synthesis of a complementary DNA strand; and wherein each oligonucleotide primer is selected to anneal to its complementary template upstream of any

labeled oligonucleotide annealed to the same nucleic acid strand;

(c) amplifying the target nucleic acid sequence employing a nucleic acid polymerase having 5' to 3' nuclease activity as a template-dependent polymerizing agent under conditions which are permissive for PCR cycling steps of (i) annealing of primers and labeled oligonucleotide to a template nucleic acid sequence contained within the target sequence, and (ii) extending the primer wherein said nucleic acid polymerase synthesizes a primer extension product while the 5' to 3' nuclease activity of the nucleic acid polymerase simultaneously releases labeled fragments from the annealed duplexes comprising labeled oligonucleotide and its complementary template nucleic acid sequences, thereby creating detectable labeled fragments; and

(d) detecting and/or measuring the signal generated by the hydrolysis of the labeled oligonucleotide to determine the presence or absence of the target sequence in the sample."

"40. A kit for detecting a target nucleic acid sequence in a sample comprising:

(a) at least one labeled oligonucleotide containing a sequence complementary to a region of the target nucleic acid, wherein said labeled oligonucleotide anneals within the target nucleic acid sequence bounded by the oligonucleotide primers of part (b) and wherein the 3' end of the labeled oligonucleotide is blocked to prevent extension by a nucleic acid polymerase having 5' to 3' nuclease activity, through which 5' to 3' nuclease activity labeled fragments from annealed

duplexes comprising labeled oligonucleotide and its complementary template nucleic acid sequence are released during a PCR amplification process; wherein the labeled oligonucleotide comprises first and second labels wherein the first label is separated from the second label by a nuclease susceptible cleavage site and wherein the labels in the labeled oligonucleotide comprise a pair of interactive signal-generating labels positioned on the oligonucleotide to quench the generation of detectable signal;

(b) a set of oligonucleotide primers, wherein

a first primer contains a sequence complementary to a region in one strand of the target nucleic acid sequence and primes the synthesis of an extension product, and a second primer contains a sequence complementary to a region in a second strand of the target nucleic acid sequence or complementary to a region in the extension product of the first primer and primes the synthesis of a complementary DNA strand;

and wherein each oligonucleotide primer is selected to anneal to its complementary template upstream of any labeled oligonucleotide annealed to the same nucleic acid strand."

"50. A reaction mixture for detecting a target nucleic acid sequence in a sample which reaction mixture comprises prior to amplification a sample, a nucleic acid polymerase having a 5' to 3' nuclease activity, a pair of oligonucleotide primers and at least one labeled oligonucleotide, which pair of primers and labeled oligonucleotide are characterized in that:

a) the labeled oligonucleotide contains a sequence complementary to a region of the target nucleic acid and anneals within the target nucleic acid bounded by the oligonucleotide primers of part b) and from which oligonucleotide through the 5' to 3' nuclease activity of a polymerase labeled fragments from annealed duplexes comprising labeled oligonucleotide and its complementary template nucleic acid sequence are released during a PCR amplification process;

b) the pair of oligonucleotide primers comprises a first primer containing a sequence complementary to one strand of the target nucleic acid and which primes the synthesis of an extension product, and a second primer containing a sequence complementary to a region in a second strand of the target nucleic acid sequence or complementary to a region in the extension product of the first primer and primes the synthesis of a complementary DNA strand;

and wherein each oligonucleotide primer is selected to anneal to its complementary template upstream of any labeled oligonucleotide annealed to the same nucleic acid strand; wherein the labeled oligonucleotide comprises a first and second label wherein the first label is separated from the second label by a nuclease susceptible cleavage site and wherein the labels in the labeled oligonucleotide are a pair of interactive signal generating labels positioned on the oligonucleotide to quench the generation of detectable signal."

Dependent claims 2 to 12, 14 to 39, 41 to 49 and 51 to 54 related to specific embodiments of the process of claim 1, the amplification process of claim 13, the kit

of claim 40 or the reaction mixture of claim 50, respectively. Dependent claim 55 covered a further embodiment of the process of claim 1 or 13.

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

(D2) EP-A-0 063 879;

(D3) Morrison L.E. et al., *Analytical Biochemistry*,
Vol. 183, pages 231-244 (1989);

(D6) Cardullo R.A. et al., *Proc. Natl. Acad. Sci. USA*,
Vol. 85, pages 8790-8794 (1988);

(D7) EP-A-0 334 694;

(D8) US-A-4,876,335;

(D9) US-A-4,780,405;

(D10) EP-A-0 258 017;

(D12) US-A-4,656,127;

(D13) Gelfand D.H., "Taq DNA Polymerase" in *PCR Technology, Principles and Applications for DNA Amplification*, Erlich Editor, Stockton Press, New York, pages 17-22 (1989);

(D18) Nelson P.S. et al., *Nucleic Acids Research*,
Vol. 17, No. 18, pages 7187-7194 (1989);

(D22) Zuckermann R. et al., Nucleic Acid Research,
Vol. 15, No. 13, pages 5305-5321 (1987);

(D23) Gupta K.C. et al.; Tetrahedron Letters, Vol. 31,
No. 17, pages 2471-2474 (1990).

VI. The submissions by appellant I (patentee), insofar as they are relevant to the present decision, can be summarized as follows:

Novelty

Claims 1 and 13

- There was no evidence that for the reaction conditions described in document (D3), the nuclease activity would serve to cleave the annealed labeled oligonucleotide to release labeled fragments as required by these claims. Furthermore, even if this should have happened this would not have served to detect a target sequence in the method of document (D3), as there would be no significant signal change by hydrolysis of one of the hybridized probes because the fluorophore label on the probe was already separated from its corresponding quencher, and hydrolysis would merely remove the label from the duplex into solution where it would continue to fluoresce.

Inventive step

Claims 1 and 13

- Although document (D13) described the general properties of *Taq* polymerase, it did not describe

the hydrolysis of modified oligonucleotide probes. According to page 19, 3rd paragraph of this document, *Taq* DNA polymerase only had a very low 5' to 3' exonuclease activity. Moreover, document (D13) did not disclose how to use that activity for any nucleic acid detection, nor whether modified oligonucleotides were degraded by this activity. Therefore, an expert reading document (D13) was certainly not motivated to use the 5' to 3' exonuclease activity in order to establish an alternative detection method, to be used e.g. instead of the detection system of document (D12).

- Moreover, even assuming that document (D13) taught that degradation of the ³²P-labeled oligonucleotide occurred, the degradation could not have been detected homogenously and the signal would not have been any different in the cleaved and uncleaved state.

- The methods of claims 1 and 13 required that the polymerase used be very specific as regards the 5'-3'-exonuclease activity, i.e. it had to hydrolyze the labeled oligonucleotide probe only when at least a portion was hybridized to the target nucleic acid and the level of its 5'-3'-exonuclease activity had to be sufficient for the level of its polymerase activity. At the priority date of the patent in suit, it was entirely unknown whether the *Taq* polymerase described in document (D13) actually had these characteristics. Document (D13) provided no hint in the direction of the claimed invention and, even if someone had had some idea on the lines of the methods of

claims 1 and 13, it would have been totally unexpected that this would actually work.

Claim 40

Inventive step

- There was no pointer in document (D3) to the specific combination of claim 40 including a pair of primers together and a dual labeled probe, as document (D3) did not teach using dual labeled probes in conjunction with a competitive hybridisation assay.

- There were at least three differences required to be present in the dual labeled probe of the kit of claim 40 compared to the assay described in document (D3), namely:
 - (i) a pair of interactive signal generating labels on the same single oligonucleotide strand of DNA which interact to quench the generation of detectable signal, whereas in document (D3) labels on the same strand do not interact;
 - (ii) the first label is separated from the second label by a nuclease susceptible cleavage site;
 - (iii) a 3'-blocking group is present. Neither document (D3) by itself, nor in combination with other documents would have led the skilled person to providing a kit with such dual labeled probes exhibiting feature (i) above.

Claim 50

Article 123(2) EPC

- The wording "a nucleic acid polymerase having a 5' to 3' nuclease activity" could be derived from page 7, lines 6 to 15 of the WO application.

Inventive step

- There was no pointer in document (D3) to the specific combination of claim 50 including inter alia a dual labeled probe, as document (D3) did not teach using dual labeled probes in conjunction with a competitive hybridisation assay.

- There were at least two differences required to be present in the dual labeled probe of the mixture of claim 50 compared to the assay described in document (D3), namely:
 - (i) a pair of interactive signal generating labels on the same single oligonucleotide strand of DNA which interact to quench the generation of detectable signal, whereas in document (D3) labels on the same strand do not interact; and
 - (ii) the first label is separated from the second label by a nuclease susceptible cleavage site.Neither document (D3) by itself, or in combination with other documents would have led the skilled person to providing a reaction mixture with such dual labeled probes exhibiting feature (i) above.

- The reaction mixture of claim 50 had to be capable of further amplification, whereas this was not true of the reaction mixture of document (D18), so

this document would not have given the skilled person any reason to arrive at the subject matter of claim 50.

VII. The submissions by appellant II (opponent), insofar as they are relevant to the present decision, can be summarized as follows:

Novelty

Claims 1 and 13

- The methods of claims 1 and 13 were the inevitable result of carrying out the method described in document (D3), according to which, a target oligonucleotide was amplified in a PCR reaction using a pair of primers. At that point, a labeled probe was added to the reaction mixture, which was then heated up to a temperature of 94°C to denature the strands, diluted with a buffer and cooled to 40°C, after which the fluorescent signal was monitored.

- The reaction mixture thus contained all the components necessary for performing the methods of claim 1 and claim 13, namely the amplified product, the labeled probe, the *Taq* polymerase and an excess of primers and bases. Under the conditions given, the labeled probe hybridized to the target between the primer sequences and a further round of primer annealing and extension took place, which would cause hydrolysis of any hybridized labeled probe by the *Taq* polymerase and release of labeled fragments to occur, as required by claim 1 at issue, or would cause said hydrolysis combined

with a further amplification step to take place,
as required by claim 13 at issue.

Inventive step

Claims 1 and 13

- The only difference between the process of claim 1 at issue and that described on page 19, lines 25-31 of document (D13) was the incorporation of a label in the probe. The problem to be solved therefore was one of adapting the process of document (D13) in order to obtain an assay based on an alternative detection system. The solution, to add a label to facilitate the detection of the digestion products which is described in document (D13) was a trivial step, which could not give rise to an inventive step.

- As for claim 13, document (D13) was a textbook reference for anybody working in the field of PCR, and thus it taught the skilled person not only that *Taq* DNA polymerase had 5'-3'-exonuclease activity and that the enzyme digested a 3'-blocked DNA probe hybridized to a target DNA sequence downstream of a primer to release fragments that were then detectable, but also that said 3'-blocked DNA probe could not be extended during a PCR reaction. Departing from document (D13) as the closest prior art, the problem to be solved was adapting the process of document (D13) in order to obtain a PCR assay based on an alternative detection system. The solution proposed, namely that of labelling the probe and then detecting the digestion products, would be obvious in view of

the passage in document (D13) which suggested labelling an oligonucleotide, the more so as document (D12) showed that detection systems could be based upon digestion of labeled probes.

- PCR amplification was known from document (D10), document (D12) or document (D3). On the basis of e.g., document (D10) as closest prior art, teaching that labeled probes could be used to detect the amplification product (see page 13, lines 44-47), the problem to be solved was one of providing an alternative detection system for the PCR assay. However, the subject-matter of claims 1 and 13 lacked inventive step in view of document (D10), document (D12) or document (D3) taken in combination with document (D13) since the latter document disclosed that *Taq* polymerase had a 5'-3'-exonuclease activity which hydrolysed a 3'-blocked probe annealed to a target DNA downstream of the primer to release fragments which were then detectable.

Claim 40

Inventive step

- The closest prior art was represented by document (D3), disclosing all the elements of the kit of claim 40, were it not for the possibility that the 3'-blocking group in the dual labeled probe described in document (D3) could be missing. Packaging these components to make a kit for carrying the homogeneous competitive hybridisation assay disclosed in document (D3) was thus obvious.

- The problem to be solved was to provide an alternative kit to that of document (D3). However, 3'-blocking of DNA probes by phosphorylation or by labelling was common practice (see documents (D2), (D6), (D7), (D8), (D9), (D18), (D22) or (D23)).
- Therefore, the skilled person could equally well select a probe cited in these documents, as the selection of such 3'-blocked probes was arbitrary in the context of the method of document (D3), resulting in the kit of claim 40.

Claim 50

Article 123(2) EPC

- Since page 7, line 16 and all the Examples of the WO application related to *Taq* polymerase, the wording in claim 50 "a nucleic acid polymerase having a 5' to 3' nuclease activity" represented added subject-matter.

Inventive step

- The arguments submitted in relation to the kit of claim 40 also applied, *mutatis mutandis*, to the reaction mixture of claim 50.

VIII. Appellant I (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of claims 1 to 55 submitted as main request at the oral proceedings on 24 May 2005, and the description and figures as granted.

Appellant II (opponent) requested that the decision under appeal be set aside and that the European patent No. 0 543 942 be revoked.

Reasons for the Decision

Claims 1 and 13

Novelty

1. Appellant II argues that claims 1 and 13 lack novelty vis-à-vis document (D3), because at the end of the process described in document (D3) (see, page 235, l-h column, second full paragraph to r-h column, line 4) there would be a reaction mixture containing the amplified product, the primers, a polymerase and a labeled probe, in which a reaction corresponding to the process of claims 1 and 13 might occur. According to appellant II, the primer and the labeled probe in this mixture would anneal to the target DNA and a further amplification cycle would occur, during which the 5'-3'-exonuclease activity of *Taq* polymerase would cause some hydrolysis of the labeled probe which is annealed to the target DNA sequence. It is further argued by appellant II that the fluorescence signals determined according to document (D3) would include a contribution from hydrolyzed labeled probe components.
2. However, there is no evidence before the board that under the experimental conditions described in document (D3), a process corresponding to the methods of claims 1 and 13, as argued by appellant II, actually occurs, nor is it plausible that it would occur. This is because the signal generating step described in the

paragraph bridging the l-h and r-h column at page 235 of document (D3) is performed under different reaction conditions (the polymerase and the divalent cation necessary for enzymatic activity have been diluted, stringency is lower (40°C instead of 37°C) and the salt composition/concentrations (1 M NaCl, 10 mM Tris/pH 8.0) have been altered from those normally used in a PCR amplification step (see e.g., page 11, line 9 of the patent in suit: 50 mM KCl, 10 mM Tris-HCl/pH 8.3, 3 mM MgCl₂).

3. But even if there were evidence supporting the view of appellant II that there would actually be some residual elongation activity and probe hydrolysis, not all requirements of claim 1 and 13 would be met.

4. The methods of claims 1 and 13 both require as a final step "detecting and/or measuring **the signal generated by the hydrolysis of the labeled oligonucleotide**" (emphasis by the board), i.e., the signal generated by the label released after hydrolysis has to be detected and/or measured and no measures to detect such a signal are described in document (D3).

5. For comparison purposes it must be noted that according to the patent while the generation of the signal occurs homogeneously, its detection/measure may be either heterogeneous, e.g., by taking at any time a sample from the reaction mixture, making an electrophoresis and seeing whether there are labeled degradation products of the probe (see e.g., paragraphs [0079] and [0080] of the patent in suit), or homogeneous, by applying the technique described in paragraphs [0067] and [0068] on page 8 of the patent.

6. According to the section of document (D3) headed "Competitive hybridization assay procedures" on page 235, a PCR-amplified double-stranded target DNA is mixed with a double-stranded oligonucleotide probe containing juxtaposed labels in each strand at the 5'-end of the one strand and at the 3'-end of the second complementary strand (cf. "1:1 complementary probe strands"). These vicinal labels form a FRET (Fluorescence Resonance Energy Transfer) pair, wherein a fluorescent reporter signal (F) is quenched by a fluorescence quencher (Q) (see the abstract on page 231, wherein reference is made to fluorescein as a fluorescent reporter signal and pyrenebutyrate or sulforhodamine 101 as a quencher). After denaturation, the mixture is cooled down and the double-stranded quenched oligonucleotide probes get separated upon binding to the complementary target DNA strands, whereby the fluorescent reporter signal is no longer quenched and a fluorescence signal is generated. The signal is thus generated by separation of the FRET-labeled oligonucleotide pair (F-Q). Otherwise stated, the detection of the target sequence is based on the measurement of the label of the hybridised probe (i.e., the reporter probe, not the quencher probe) but **not** on detecting/measuring the signal generated by the labeled degradation products of the probe as required by claims 1 and 13.
7. It is argued by appellant II that the fluorescence signal measured according to document (D3) would include a contribution from hydrolyzed labeled probe components. However, even assuming that some residual hydrolysis of the labeled probe takes place, this would

remain a "hidden" technical effect in the sense that it would fail to translate into any significant additional signal variation over the signal change caused by the preceding hybridisation (and separation of the F-Q pair). This is because once the fluorescent label (F) on the probe is separated from its corresponding quencher (Q), it starts to fluoresce, whether it is bound to the duplex, or whether it is released by hydrolysis from the duplex into the surrounding solution in the form of hydrolyzed labeled probe components. In other words, a signal caused by hybridisation of the labeled probe to the target DNA, possibly including a contribution from hydrolyzed labeled probe components, is indistinguishable from one not including said contribution. Thus it cannot be said that a signal contributed by the hydrolyzed probe components is measured as a means to detect a target nucleic acid sequence in the sample. Under these circumstances, it cannot be concluded that document (D3) directly and unambiguously teaches the skilled person to detect/measure the signal generated by the labeled degradation products of the probe as required by claims 1 and 13, let alone that document (D3) teaches doing this in order to establish a link between this effect and detection of a target DNA.

8. In conclusion, the subject-matter of claims 1 and 13 satisfies the requirements of Article 54 EPC.

Inventive step

9. In a first line of argument, appellant II relies on document (D13) alone for questioning the inventive step of claims 1 and 13.

10. As regards claim 1, it is argued that the only difference between the process of claim 1 at issue and that described on page 19, lines 27-33 of document (D13) is the incorporation of a label in the probe in the method of claim 1, bearing in mind that claim 1 does not relate to PCR amplification as such, but to a process for detecting a target nucleic acid which involves a single extension phase, merely requiring that a sample containing a single-stranded nucleic acid be contacted with a primer and a labeled probe, in the presence of a polymerase.

11. As for claim 13, appellant II points out that document (D13) is a textbook reference for anybody working in the field of PCR, and thus it teaches the skilled person not only that *Taq* DNA polymerase had 5'-3'-exonuclease activity and that the enzyme digests a 3'-blocked DNA probe hybridized to a target DNA sequence downstream of a primer to release fragments that are then detectable, but also that said 3'-blocked DNA probe could not be extended during a PCR reaction.

12. Departing from document (D13) as the closest prior art, in the view of appellant II, the problem to be solved was adapting the process of document (D13) to an assay (claim 1) or a PCR assay (claim 13) based on an alternative detection system. The solution proposed, namely that of labelling the probe and then detecting the digestion products, would be obvious in view of the passage in document (D13) which suggests labelling an oligonucleotide and observing digestion or degradation of the oligonucleotide as a measure of exonuclease activity (see page 19, lines 24-27: "*Taq* DNA polymerase

has a DNA synthesis-dependent strand replacement 5'-3'-exonuclease activity. There is little, if any degradation of a 5' ³²P-labelled oligodeoxynucleotide, either as single-stranded DNA or where annealed to an M13 template."), the more so as references such as document (D12) show that detection systems could be based upon digestion of labeled probes.

13. The passage on page 19, lines 27-33 of document (D13), which, according to appellant II, teaches the skilled person that Taq DNA polymerase has 5'-3'-exonuclease activity and that the enzyme digests a 3'-blocked DNA probe hybridized to a target DNA sequence downstream of a primer to release fragments that are then detectable, reads:

"Furthermore, the presence of a "blocking", annealed, non-extendable oligodeoxynucleotide "primer" (3'-phosphorylated during synthesis) fails to attenuate incorporation from a 3'-OH terminated upstream primer. There is little, if any, displaced "blocking primer", and the products of exonuclease action are primarily deoxynucleoside monophosphate (85%) and dinucleoside phosphate (15%, S. Stoffell, unpublished)."

14. However, the board observes that lines 25-27 of this passage state that Taq polymerase has a very low 5'-3'-exonuclease activity for the cited 5'-³²P-oligonucleotide annealed to an M13 template (c.f. "There is little, if, any,..."). Therefore, the skilled person reading that part of document (D13) is motivated neither to use 5'-3'-exonuclease activity nor modified probes (be they 5'-³²P-modified or otherwise) for

- nucleic acid detection in order to establish an alternative detection method.
15. More importantly, even assuming that document (D13) actually teaches the skilled person that *Taq* polymerase digests a 5'-labeled DNA probe hybridized to a target DNA sequence downstream of a primer to release labeled fragments that are then detectable (which is not the view taken by the board), there would still remain in document (D13) a missing link between these properties/behaviour of *Taq* polymerase and taking advantage of these technical effects for making an assay for detecting a target DNA sequence.
 16. Appellant II maintains that document (D12) encouraged the skilled person to turn to detection systems based upon the digestion of labeled probes. However, a skilled person coming across document (D12) is taught that the detection system described in this document (see column 4, lines 51-59) is based upon digestion of labeled probes by means of Exonuclease III, an enzyme which digests double-stranded nucleic acids from the 3'-end. Therefore, this technical teaching would rather point to the opposite direction than using the 5'-3'-exonuclease activity of *Taq* DNA polymerase in order to establish an alternative detection method.
 17. In view of the foregoing, the board concludes that the skilled person departing from document (D13) alone or in combination with document (D12) would not arrive at the methods of claim 1 or 13 in an obvious manner.
 18. In a further line of argument, appellant II maintains that the subject-matter of claims 1 and 13 lacks

- inventive step in view of document (D10), document (D12) or document (D3) taken in combination with document (D13). It is argued that departing from e.g., document (D10) as closest prior art, which document teaches that labeled probes can be used to detect the amplification product of PCR (see page 13, lines 44-47), the problem to be solved is one of providing an alternative detection system for the PCR assay. However, the skilled person would turn to document (D13), disclosing that *Taq* polymerase has a 5'-3'-exonuclease activity which hydrolyses a 3'-blocked probe annealed to a target DNA downstream of the primer to release fragments which are then detectable.
19. The board agrees that one of documents (D10), (D12) or (D3) represents the closest prior art since it deals with detecting target DNAs (see page 13, lines 44-47), the problem to be solved being one of providing an alternative detection system.
20. However, as already emphasized under point 14 supra, a skilled person coming across document (D13) is not motivated to use the 5' to 3'-exonuclease activity in order to establish an alternative detection method because he/she is taught that this activity is "very low, if any", and moreover it cannot be derived from this document whether modified oligonucleotides (be they 5'-³²P-modified or otherwise) are degraded by this activity.
21. In conclusion, the subject-matter of claims 1 and 13 also satisfies the requirements of Article 56 EPC.

Claim 40

Articles 123(2) and 54 EPC

22. Claim 40 is directed to a kit for detecting a target nucleic acid sequence in a sample comprising one labeled oligonucleotide (labeled probe) and a set of oligonucleotide primers. The claim now incorporates the features of granted claim 49, namely that "said labeled oligonucleotide comprises first and second labels wherein the first label is separated from the second label by a nuclease susceptible cleavage site, and granted claim 50, namely that "the labels in the labeled oligonucleotide comprise a pair of interactive signal-generating labels positioned on the oligonucleotide to quench the generation of detectable signal" (see section IV supra). No objections under the above Articles have been raised by appellant II and the board also sees none.

Inventive step

23. Appellant II maintains that the closest prior art underlying the kit of claim 40 is represented by document (D3), which discloses a set of oligonucleotide primers and a 5'-F, 3'-Q-dual labeled probe as separate elements and that it was obvious to package these separate components to make a kit for carrying the homogeneous competitive hybridisation assay disclosed in document (D3). It is further argued by appellant II that if any difference turned up between the so obtained kit and that covered by claim 40, this could only lie in the 3'-blocking group missing in the 5'-F, 3'-Q dual labeled probe described in document (D3), however, the skilled person wishing to provide an

alternative kit to that of document (D3) for carrying the assay disclosed therein could equally well select a 3'-blocked probe cited in documents (D2), (D6), (D7), (D8), (D9), (D18), (D22) or (D23), arriving at the kit of claim 40 in an obvious manner.

24. Document (D3) indeed describes a method of detecting a PCR amplified DNA sequence in a sample by using a competitive hybridisation assay involving two complementary probes, one of which is labeled at the 5'-end with a fluorophore reporter group (F), whereas the second probe is labeled at the 3'-end with a quencher (Q). Alternatively a dual labeled probe can be used, where there are a 5'-F and a 3'-Q on the same strand, which are hybridised to a complementary dual 5'-F, 3'-Q-labeled strand to form a duplex exhibiting four labels, wherein each pair (F-Q) is quenched. The legend to Fig. 6 on page 240 of document (D3) in fact illustrates such an approach for the detection of the E. coli enterotoxin gene in a sample. Five complementary pairs of labeled probes are used simultaneously to increase sensitivity. However, focusing on one of these five probes, each strand of the complementary pairs is labeled with both a 5'-fluorescein (F) and a 3'-pyrenebutyrate (Q). The idea behind this technique is that the resting state of the system (i.e., the duplex with four labels) has quenched fluorescence, but once a target sequence is introduced or present in sufficient quantity, e.g. following PCR amplification, the signal is turned on due to the removal of the quenching through separation of the strands (and hence of the F-Q pairs) upon hybridisation. The board thus agrees that document (D3) discloses a set of oligonucleotide

- primers and a 5'-F, 3'-Q-dual labeled probe as separate elements.
25. However, in the 5'-F, 3'-Q-dual labeled probe described in document (D3), the labels positioned on the **same** oligonucleotide strand are **not interactive** (unlike those positioned on the complementary DNA strands), i.e., they are not and cannot be vicinal. Otherwise, there would be no generation of signal upon hybridization, as no removal of the quenching through separation of the strands (and hence of the F-Q pairs) would occur: it does not make sense that the labels positioned on the same oligonucleotide strand be vicinal. This view is supported by page 240, 1-h column of document (D3): four 5'-F, 3'-Q-labeled probe pairs were 21 bases in length and the fifth pair was 22 bases in length.
26. To the contrary, a critical feature of the labeled probe of the kit of claim 40 lies in the requirement that the pair of signal-generating labels positioned on one and same oligonucleotide strand must be interactive (vicinal) to quench the generation of detectable signal. This is because the signal has to be turned on by hydrolysis of the probe by the 5' to 3' nuclease activity of the nucleic acid polymerase (see paragraph [0067] of the patent in suit and claim 29 at issue).
27. As a consequence, the 5'-F, 3'-Q-dual labeled probe of the kit of claim 40 is **not** suitable for carrying out the method described in document (D3).
28. Therefore, regardless of whether or not the skilled person would package the separate components disclosed in document (D3) (i.e., a set of oligonucleotide

primers and a 5'-F, 3'-Q-dual labeled probe) and optionally 3'-block the 5'-F, 3'-Q dual labeled probe for carrying the assay disclosed document (D3) (see point 23 supra), there would already be a technical blockage preventing the skilled person from arriving at a pair of interactive signal-generating labels positioned on the labeled oligonucleotide probe to quench the generation of detectable signal, and thus the kit of claim 40 cannot be derived in an obvious way from the prior art.

Claim 50

Article 123(2) EPC

29. Claim 50 relates to a reaction mixture which comprises a sample, a nucleic acid polymerase having a 5' to 3' nuclease activity, a pair of oligonucleotide primers and at least one labeled oligonucleotide. Compared with the corresponding claim as granted, the claim now incorporates the features "a sample", "a nucleic acid polymerase having a 5' to 3' nuclease activity" and the features of granted claim 59, namely that "said labeled oligonucleotide comprises first and second labels wherein the first label is separated from the second label by a nuclease susceptible cleavage site, and granted claim 60, namely that "the labels in the labeled oligonucleotide comprise a pair of interactive signal-generating labels positioned on the oligonucleotide to quench the generation of detectable signal" (see section IV supra).
30. The reaction mixture of claim 50 is the result of performing steps (a), (b) and (c) (see page 3, lines 1 to 14 of the published WO application), however, before

switching to "conditions which are permissive for PCR cycling steps" (*ibidem*, line 14). Claim 50 thus finds a basis in published WO application.

31. The only objection under Article 123(2) EPC raised by appellant II is that since page 7, line 16 and all the Examples of the WO application relate to Taq polymerase, the wording in claim 50 "a nucleic acid polymerase having a 5' to 3' nuclease activity" represents added subject-matter. However, it can be derived from page 7, lines 6 to 15 of the WO application that any polymerase can be used "if possessing a 5' to 3' nuclease activity" and that Taq polymerase exhibits such activity.

32. Therefore, claim 50 meets the requirements of Article 123(2) EPC.

Article 54 EPC

33. No objection of lack of novelty has been raised by appellant II and the board also sees none.

Inventive step

34. Claim 50 is directed to a reaction mixture for detecting a target nucleic acid sequence. In particular this reaction mixture comprises at least one labeled oligonucleotide complementary to a region of the target nucleic acid, which oligonucleotide comprises a pair of interactive signal generating labels which interact to quench the generation of detectable signal.

35. The conclusion arrived at by the board in relation to the kit of claim 40 that there was a technical blockage preventing the skilled person from arriving at such labeled oligonucleotide probe including a pair of interactive signal-generating labels positioned on the same DNA strand to quench the generation of detectable signal, also applies to the reaction mixture of claim 50, which thus cannot be derived in an obvious way from the prior art, either.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The matter is remitted to the first instance with the order to maintain the patent on the basis of claims 1 to 55 submitted as main request at the oral proceedings on 24 May 2005, and the description and figures as granted.

Registrar:

Chair:

P. Cremona

U. M. Kinkeldey