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Datasheet for the decision of 20 October 2014

Case Number: T 2533/10 - 3.3.08

Application Number: 98108491.6

Publication Number: 0878554

IPC: C12Q1/68

Language of the proceedings: EN

Title of invention:
Detection of nucleic acids by fluorescence quenching

Patent Proprietor:
Becton Dickinson and Company

Opponent:
The Secretary of State for Defence
Directorate of Intellectual Property Rights

Headword:
Amplification quenched fluorophobes primer probes restriction endonuclease recognition site/BECTON DICKINSON

Relevant legal provisions:
EPC Art. 123(2), 83, 54(3), 56
RPBA Art. 13(1)

Keyword:
Main Request - admissibility (yes); added subject-matter (no); sufficiency of disclosure (yes); novelty (yes); inventive step (yes)

Decisions cited:
T 0019/90

Catchword:

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Case Number: T 2533/10 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 20 October 2014

Appellant: (Patent Proprietor)	Becton Dickinson and Company One Becton Drive Franklin Lakes, NJ 07417-1880 (US)
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Appellant: (Opponent)	The Secretary of State for Defence Directorate of Intellectual Property Rights 2 Poplar nr 2218 MoD Abbey Wood Bristol BS34 8JH (GB)
Representative:	Beckham, Robert William Ministry of Defence Defence Intellectual Property Rights Oak 2E, Nr. 6228 Abbey Wood Bristol BS324 8JH (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 2 November 2010 concerning maintenance of the European Patent No. 0878554 in amended form.**

Composition of the Board:

Chairman	M. Wieser
Members:	P. Julià
	J. Geschwind

Summary of Facts and Submissions

I. An opposition was filed against European patent 0 878 554 (based on European patent application 98 108 491.6, with filing and priority dates of 11 May 1998 and 13 May 1997, respectively) on the grounds of Articles 100(a),(b) and (c) EPC.

Claim 8 of the Main Request (claims as granted) was considered by the opposition division to contravene the requirements of Article 123(2) EPC and claim 8 of Auxiliary Request 1 (filed on 8 April 2010) was found not to meet the requirements of Article 54(3) EPC. The opposition division maintained the patent on the basis of Auxiliary Request 2 filed on 9 June 2010.

II. Appeals were lodged by the patentee (appellant I) and by the opponent (appellant II) against the decision of the opposition division. With the statement of Grounds of Appeal, appellant I maintained its Main Request and Auxiliary Requests 1 and 2.

III. With its reply to appellant II's Grounds of Appeal, appellant I filed Auxiliary Requests 2a and 3-10 and raised an objection against the admissibility of appellant II's "Notice of Appeal". Appellant II replied to appellant I's Grounds of Appeal.

IV. In a communication pursuant to Article 15(1) of the Rules of procedure of the Boards of Appeal (RPBA), annexed to the summons to oral proceedings, the appellants were informed of the preliminary opinion of the board on the issues of the case.

V. In reply to the board's communication, appellant I maintained its Main Request and Auxiliary Requests 1-2,

withdrew former Auxiliary Requests 2a and 3-10, and filed new Auxiliary Requests 3-8.

VI. Appellant II did not file substantive submissions in reply to the board's communication but announced its intention to attend the oral proceedings.

VII. Oral proceedings were held on 20 October 2014. At these proceedings, appellant I withdrew the objection raised against the admissibility of appellant II's "Notice of Appeal" and made its former Auxiliary Request 4 to its new Main Request. The former Main Request (claims as granted) and Auxiliary Requests 1-3 were all withdrawn.

VIII. Claims 1, 3 and 7 of the new **Main Request** read as follows:

"1. A method for detecting presence of a target sequence comprising:

a) hybridizing to the target sequence a signal primer comprising a target binding sequence and a restriction endonuclease recognition sequence 5' to the target binding sequence, the restriction endonuclease recognition sequence flanked by a donor fluorophore and an acceptor dye such that fluorescence of the donor fluorophore is quenched;

b) in a primer extension reaction, synthesizing a complementary strand using the signal primer as a template and the target sequence as the primer, thereby rendering the restriction endonuclease recognition sequence double-stranded;

c) cleaving or nicking the double-stranded restriction endonuclease recognition sequence with a restriction endonuclease, thereby reducing donor fluorophore quenching and producing a change in a fluorescence parameter, and;

d) detecting the change in the fluorescence parameter as an indication of the presence of the target sequence."

"3. A method for detecting amplification of a target sequence comprising, in an amplification reaction:

a) hybridizing to the target sequence a first primer comprising a target binding sequence and a restriction endonuclease recognition sequence 5' to the target binding sequence, the restriction endonuclease recognition sequence flanked by a donor fluorophore and an acceptor dye such that fluorescence of the donor fluorophore is quenched;

b) extending the hybridized first primer on the target sequence with a polymerase to produce a first primer extension product having an extended portion and separating the first primer extension product from the target sequence;

c) rendering the separated first primer extension product and the restriction endonuclease recognition sequence double-stranded by hybridization and extension of a second primer which hybridizes to the extended portion of the first primer extension product;

d) cleaving or nicking the double-stranded restriction endonuclease recognition sequence with a restriction endonuclease, thereby reducing donor fluorophore

quenching and producing a change in a fluorescence parameter, and;

e) detecting the change in the fluorescence parameter as an indication of amplification of the target sequence."

"7. A single-stranded oligonucleotide comprising:

(a) a target binding sequence;

(b) a restriction endonuclease recognition site 5' to the target binding sequence, wherein all of the restriction endonuclease recognition site remains single stranded upon hybridization of the oligonucleotide to the target sequence, and;

(c) a first dye and a second dye linked to the oligonucleotide at positions flanking the restriction endonuclease recognition site such that fluorescence of the first or the second dye is quenched,

wherein the first and second dyes are 8-20 nucleotides apart in the oligonucleotide."

Whilst claim 2 was directed to a preferred embodiment of claim 1, claims 4-6 and 8 were directed to preferred embodiments of claims 3 and 7, respectively.

IX. The following documents are cited in this decision:

D1: G.T. Walker et al., Molecular and Cellular Probes, 1995, Vol. 9, pages 399 to 403;

D2: US 5,538,848 (publication date: 23 July 1996);

D9: EP-B1-0 912 597 (filing date of the application: 15 July 1997; first priority date: 16 July 1996).

X. Appellant I's (patentee's) submissions, insofar as they are relevant to the present decision, are summarized as follows:

Admissibility of the Main Request

The Main Request was originally filed as Auxiliary Request 4 in reply to the board's communication. The amendments introduced into the request addressed the objections raised in this communication, some of them were new in the proceedings. These amendments were straightforward and consisted of the deletion of contentious subject-matter and the introduction of features of a dependent claim into an independent claim.

Main Request
Article 100(c) EPC; Article 123(2) EPC
Claims 1-2 and 3-6

The subject-matter of granted claim 2 had been deleted. Claims 1-2 had a basis in the application as filed. Claim 3 was based on original claim 4 which described a method for target dependent amplification that did not require an upstream primer. The signal primer was extended so as to have an extended portion which was subsequently bound to the second primer and thereby, a signal was detected. According to the application as filed, the amplification primers could be labeled and modified as described for signal primers (page 16, lines 31-38). The first primer in the method of claim 3 could be the labelled amplification primer and its extended portion could then bind to the second primer.

Claim 3 was not limited to a strand displacement amplification (SDA) method but could be adapted to other primer extension amplification methods (page 15, lines 32-33). If, in these methods, a labelled amplification primer was used, the displacement of the extended labeled primer could occur by using bumper primers, heating or enzymatic digestion (page 16, line 33 to page 17, line 1).

Article 100(b) EPC; Article 83 EPC
Claim 7

Table I of the patent showed that separations of 9 and 14 nucleotides between donor and acceptor fluorophore dyes provided good fluorescence signals while sub-optimal results were obtained with 4 and 24 nucleotides and the best signals were provided with a separation of 11 nucleotides. Separations of 8-20, preferably 10-16, nucleotides produced a detectable change in fluorescence. Placing acceptor and donor dyes closer together than 6 nucleotides interfered with the ability of the restriction enzyme to cleave the duplex. Thus, Table I informed a skilled person that, for an optimal effect, a balance in the separation between donor and acceptor dyes had to be stricken, so as to allow the restriction endonuclease to cleave the duplex and the fluorescence quenching of the fluorophore pair to be effective. The range in claim 7 was not arbitrary but gave a window of opportunity. For a particular fluorophore pair, a skilled person could optimize the distance and thereby the fluorescence effect.

Article 100(a) EPC; Article 54(3) EPC
Claim 7

Claim 7 was directed to a single-stranded oligonucleotide, not to an oligonucleotide comprising a single-stranded region. The oligonucleotide shown in Figure 24D of document D9 was not a single-stranded oligonucleotide but a hairpin primer comprising three regions: a single-stranded region, a double-stranded stem region and a hairpin region. In Figure 24D not all of the restriction endonuclease recognition site (RERS) (which was not identified as such in document D9) was single-stranded, the first 5'(G) nucleotide hybridized with a (C) nucleotide of the stem region. Step 1 in Figure 10 of document D9 showed that the stem-loop structure did not disappear upon binding to the target sequence; the oligonucleotide was not linearized but remained partially double-stranded. Step 2 of Figure 10 showed the oligonucleotide to be fully double-stranded. Thus, feature (b) of claim 7 was not disclosed in document D9.

Article 100(b) EPC; Article 56 EPC

Contrary to the claimed homogeneous real-time detection methods, the closest prior art document D1 disclosed an end-point detection SDA method requiring a separation/ filtration step and was not relying on a probe having a fluorophore-quenched pair. The problem to be solved was the provision of a real-time detection method for a target DNA sequence in a homogeneous assay. The examples of the patent showed the claimed methods and probe to solve this problem.

There was no hint in document D1 to a real-time detection or to a detector probe with a fluorophore-quenched pair and to a RERS and a target binding site with the configuration defined in claim 7. Also a suggestion to adapt the detection system disclosed in

document D1 to such a probe could not be found. A combination of documents D1 and D2 required hindsight knowledge of the patent. The SDA and the PCR amplification methods disclosed in documents D1 and D2, respectively, were fundamentally different and relied on divergent features. The PCR method relied on temperature-dependent cycles and a DNA polymerase suitable thereto. Whilst a DNA polymerase with a 5'-3' exonuclease activity was essential for the PCR method disclosed in document D2, this activity could not be present in the DNA polymerase of the SDA method disclosed in document D1. Whilst a 3' blocked probe in the PCR method was preferred, such a blocking rendered the probe useless in the SDA method. Likewise, the introduction of a RERS (and its cleavage) in the probe would have rendered it useless for the PCR method disclosed in document D2. A cautious skilled person, as defined in the established case law ("Case Law of the Boards of Appeal of the EPO", 7th edition 2013, I.D. 8.1.3, page 189), would not have transferred features from the probe and the PCR method disclosed in document D2 to the probe and the SDA method disclosed in document D1.

Although document D1 cited fluorescence as a possible alternative to radiometric detection, this hint, if at all, would have motivated a skilled person to replace the ^{32}P-labelled probe by a fluorescence labelled probe having the same properties as the disclosed radiometric probe. Even if a skilled person would have contemplated probes with other properties, it would not have arrived in an obvious manner at a probe with the features defined in claim 7, since other probes could also be contemplated, such as probes used in fluorescence polarization detection, hairpin or double imperfect hairpin probes and the probes shown in document D9. The

selection of a probe having all the features of the probe defined in claim 7 was not straightforward. The bibliographic reference in document D1 to a PCR method using a cleaved detector probe did not lead a skilled person to document D2, even though the same reference was cited in document D2 (the "*Tac-Man*" approach). The detector probe used in the PCR method of the referred document relied on a 5' ^{32}P-labelled probe, not on a fluorophore-quenched pair. The referred document was not on file and its content was thus irrelevant to the proceedings.

There was no information in document D2, nor in any other document on file, how to achieve a suitable separation between two fluorophore dyes in a probe for having the fluorophore pair to be quenched and a restriction endonuclease to be effective in the RERS cleavage. The patent demonstrated the presence of a distance or separation range, a window of opportunity, where both effects were achieved. Without this knowledge, a skilled person could not have had any reasonable expectation of success.

XI. Appellant II's (opponent's) submissions, insofar as they are relevant to the present decision, are summarized as follows:

Admissibility of the Main Request

The Main Request was filed at a very late stage of the proceedings and no reasons were provided to explain why it could not have been filed earlier.

Main Request
Article 100(c) EPC; Article 123(2) EPC
Claims 1-2

Two alternative embodiments were disclosed in the application as filed, namely an amplification and a non-amplification method. Arrangements with a 5' overhang were associated with a non-amplification method only and, accordingly, step (b) of claim 1 was always disclosed in the context of a non-amplification method. There was no basis in the application as filed for the detection method of claim 1, if it was interpreted as comprising an amplification method, such as described in granted claim 2 which defined the complementary strand of claim 1(b) as being synthesized in a target amplification reaction.

Claims 3-6

The introduction into claim 3 of the feature "*having an extended portion*", without requiring the presence of an upstream primer, was new subject-matter not directly derivable from the application as filed. The alleged basis on page 7, line 25 *et seq.* described Figure 1. In both this figure and in its description, there was a requirement of an amplification primer or a signal primer that was upstream to the signal extension product. When extended, the upstream primer knocked the signal primer extension product off the template/target strand. Without the upstream primer, there was no explanation as to how the signal primer extension product dissociated from the target strand and bound to the second primer.

Moreover, the application as filed only described "*a signal primer extension product*" but not an extended portion as cited in claim 3. An extension product, as defined on page 6, lines 17-20 of the application as filed, included the primer or a portion thereof,

whereas the "*extended portion*" of claim 3 did not include the primer and thus added new subject-matter.

Article 100(b) EPC; Article 83 EPC
Claims 1-2 and 7

A signal primer binding to a target sequence could function as a template (and the target strand as the primer) only when the 3' end of the target strand was free to extend, i.e. the primer was bound to the target strand with a 5' overhang. If the primer was bound somewhere along the length of the target strand, there was no free 3' end available to form the complementary strand. Claim 1 did not exclude this possibility.

The disclosure of the patent was not sufficient to allow a skilled person to perform the invention without undue burden over the whole scope of claim 7. There was no limitation in claim 7 as to the type and nature (homologous, heterologous) of the fluorophore pair and the (8-20) nucleotide range given in the claim was arbitrary. Table I showed that a fluorescence effect was achieved at a distance of 11 and 16 nucleotides only for heterologous fluorophore pairs but no effect was detected for homologous fluorophore pairs at a distance of 11 nucleotides. No data was provided to show such effect for fluorophore pairs at the upper and lower limits of the range given in claim 7. Table I did not demonstrate a demarcation between presence and absence of the effect at 8 (active RERS cleavage) and 20 (effective quenching) nucleotides separation. The patent only disclosed a few embodiments, but not a range, for which the effect was achieved. The skilled person was left alone to look for suitable fluorophore pairs and to select, for each of them, an appropriate distance within the range given in claim 7 for

achieving a fluorescence effect. This amounted to undue burden.

Article 100(a) EPC; Article 54(3) EPC
Claim 7

Claim 7 was a product-claim characterized by structural features that were all present in the oligonucleotide shown in Figure 24D of document D9. This oligonucleotide was single-stranded (even though under certain conditions it had a hairpin structure), comprised a sequence complementary to a target sequence and a (*Afa*I, 5'-GTAC-3') RERS not overlapping with this complementary sequence. It remained single-stranded upon hybridization to the target sequence, as shown in step 1 of Figure 10 in document D9. The RERS was an inherent feature of the single-stranded oligonucleotide. The two fluorophore dyes in this single-stranded oligonucleotide were separated by 20 nucleotides.

Article 100(a) EPC; Article 56 EPC

The closest prior art document D1 disclosed a SDA method for detecting a target DNA sequence. This method used a signal primer (detector probe) hybridizing to a target sequence which comprised a RERS 5' to the target binding sequence (Step 1 in Figure 1). After strand displacement amplification, the uncleaved and cleaved detector probes were separated by filtration. According to document D1, the SDA/filtration system was adaptable to a closed format using non-radiometric detection, such as fluorescence detection. The problem to be solved was the adaption of this SDA/filtration method to a closed format system using fluorescence detection.

No hindsight was required in the formulation of this problem, since it was derivable from document D1.

Document D1 referred to a detection system described for PCR and using a polymerase with 5'-3' nuclease activity. The bibliographic reference given in document D1 was cited in document D2 and concerned the "*Tac-Man approach*", a well-known assay at the priority date of the patent. The "*Tac-Man*" assay provided real-time measurements of PCR amplification products by detecting the cleavage (and thereby the change from a double-stranded to a single-stranded sequence) of a probe containing a fluorophore-quenched pair. Thus, document D1 contained a link to the PCR amplification method and to the detection method disclosed in document D2. A skilled person had a motivation to amend the detector probe used in the SDA method disclosed in document D1 to a probe containing a fluorophore-quenched pair as used in the PCR amplification method disclosed in document D2. Thereby, the skilled person would have obtained in an obvious manner the probe defined in claim 7 and also its use in the methods of claims 1 and 3.

No technical problems would have been encountered when carrying out this adaption. Document D2 disclosed preferred reporter-quencher pairs and appropriate distances (about 6-16 nucleotides) between them. It further acknowledged that the precise properties of the probe had to be tailored to the nature of the target DNA sequence. Indeed, a skilled person would have easily noted that some of the optional features of the probes used in the PCR amplification method disclosed in document D2 (such as a blocked 3' terminal) as well as the properties of the DNA polymerase used in this method (such as a 5'-3'exonuclease activity) and which

were not essential for the signalling means or the detection system itself, were not to be imported to the SDA method disclosed in document D1.

XII. Appellant I (patent proprietor) requested that the decision under appeal be set aside and the patent be maintained on the basis of the Main Request (claims 1 to 8) filed on 20 October 2014 at the oral proceedings before the board.

XIII. Appellant II (opponent) requested that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Admissibility of the Main Request

1. The Main Request was originally filed as Auxiliary Request 4 in reply to the board's communication pursuant to Article 15(1) RPBA and was made the Main Request at the oral proceedings before the board (cf. points V and VII *supra*). This request represents a change of appellant I's case and, according to Article 13(1) RPBA, it lies within the board's discretion to admit it into the appeal proceedings.

2. The amendments introduced into the Main Request address objections raised in the board's communication. These amendments consist of the deletion of contentious subject-matter and of the introduction of features of a dependent claim into the corresponding independent claim. They are in line with amendments that were already present in previous auxiliary requests filed by appellant I in reply to appellant II's Grounds of Appeal. Neither the nature nor the character of these

amendments came as a surprise to appellant II and the board.

3. Thus, the board, in exercise of its discretion, decides to admit the Main Request into the appeal proceedings (Article 13(1) RPBA).

Article 100(c) EPC; Article 123(2) EPC
Claims 1-2

4. Claim 1 of the Main Request is identical to claim 1 as originally filed except for the additional feature "*and the target sequence as the primer*" in claim 1(b). The method of claim 1 is described, *inter alia*, on page 10, last paragraph of the application as filed, wherein this additional feature is explicitly referred to in lines 24-26. Claim 2 of the Main Request is identical to claim 3 as originally filed.

Claims 3-7

5. The application as filed discloses a single-stranded primer (cf. *inter alia*, page 4, lines 14-19 and claims 8-10 of the application as filed) which is used in a first embodiment of the invention, namely a method for detecting amplification of a target sequence (cf. *inter alia*, page 4, lines 28-29, page 17, Example 1, claims 4-7 of the application as filed), and in a second alternative embodiment of the invention, namely a non-amplification method for detecting a target sequence (cf. *inter alia*, page 4, lines 29-34, page 19, Example 2, claims 1-3 of the application as filed).

6. Claim 3 of the Main Request is directed to the first embodiment of the invention, i.e. a method for detecting amplification of a target sequence, and is

identical to claim 4 as originally filed except for the two additional features "*... having an extended portion ...*" and "*... which hybridizes to the extended portion of the first primer extension product*" in claim 3(b) and 3(c), respectively (cf. point VIII *supra*). These two features are directly and unambiguously derivable from the application as filed.

6.1 The method of claim 3 is shown in Figure 1 and described on page 7, lines 21-35 of the application as filed. According thereto, "... *(t)he signal primer extension product is displaced from the template by extension of the* ***upstream*** *amplification primer ...*" (emphasis added by the board). This is in line with Figure 1 and the disclosure on page 6, line 35 to page 7, line 3, wherein the signal primer is defined as hybridizing to "... *a target sequence* ***downstream*** *of an amplification primer ...*" (emphasis added by the board). Claim 4 as originally filed did not refer to the relative position of the first (signal) primer and the second (amplification) primer (S2). The amendment introduced into claim 3(b) defines the "*first primer extension product*" as "*having an extended portion*" (in line with the definition found on page 6, lines 17-19 of the application as filed) which hybridizes to "*a second primer*", as required by the amendment introduced into claim 3(c). The amendments only bring claim 4 as originally filed in line with the complete disclosure in the application as filed, as shown in Figure 1. Thus, the board considers the amendments introduced into claim 3 of the Main Request to have a clear and unambiguous, implicit basis in the application as filed.

6.2 It is also worth noting that claim 4 as originally filed, which did not require the presence of an

upstream primer, already referred to "*a first primer extension product*" in claim 4(b) and to an "*extension of a second primer*" in claim 4(c). As stated above, the amendments introduced into claims 3(b) and 3(c) of the Main Request render explicit features which were already implicitly disclosed in the application as filed, as illustrated in Figure 1 and on page 7, lines 21-35 thereof. There is no contradiction between these amendments and the definition of "*(e)xtension products*" given on page 6, lines 17-19 of the application as filed.

7. Thus, the Main Request fulfils the requirements of Article 123(2) EPC.

Article 100(b) EPC; Article 83 EPC
Claims 1-2

8. While claim 1 of the Main Request relates to a non-amplification method for detecting a target sequence (cf. page 3, paragraph [0008], lines 50-52 of the patent), claim 3 relates to an alternative method that contemplates an amplification reaction, namely the use of "*the signal primer of the invention ... in an amplification reaction for detection of target sequence amplification*" (cf. page 3, paragraph [0008], lines 49-50 of the patent). This disclosure in the "*Summary of the Invention*" is in line with the entire content of the description of the patent and the teaching conveyed thereby to a skilled person (cf. pages 10-11, Examples 1-2 of the patent). Both amplification and non-amplification methods are clearly distinguished in the patent, even though some products, such as "*the signal primer of the invention*", may be used (with or without appropriate modifications) in both methods (cf. point 5 *supra*).

9. The non-amplification based method of claim 1 is described on page 3, paragraph [0008], lines 50-52 and on page 6, paragraph [0025], lines 43-57 of the patent. On page 3, in the "*Summary of the Invention*", the signal primer is described as hybridizing at the 3' end of the target sequence so that the restriction endonuclease recognition site (RERS) forms a 5' overhang. The hybridization at the 3' end of the target sequence and the presence of such a 5' overhang is also mentioned in the "*Detailed Description of the Invention*" (cf. page 6, lines 44-45) where it is described for the two possible situations, namely when the target binding sequence of the signal primer is complementary i) to the entire length of the target sequence, or ii) to only a portion thereof (cf. page 6, lines 47-49 and lines 49-51, respectively).

10. The presence of a 5' overhang results, always and necessarily, from the defined structure of the signal primer ("*a RERS 5' to the target binding sequence*"). Although there is no explicit requirement in claim 1 for the signal primer to hybridize to the 3'-end of the target sequence, this is implicitly required by claim 1(b), which defines the target sequence as being a primer to be used in the primer extension reaction. Both features, which are essential for the alternative non-amplification method of claim 1, are thus implicitly present in claim 1. Therefore, claim 1 does not include the non-working embodiment referred to by appellant II, namely the binding of the signal primer to a target sequence "*somewhere along its length*" (cf. point XI *supra*).

Claims 7-8

11. In view of the results shown in Table I and the information in paragraph [0033] on page 8 of the patent, the board considers appellant II's objection to be without merit. Although no results are given in the patent for the upper and lower nucleotide limits of the range indicated in claim 7, the patent identifies a separation of 10-16 nucleotides between the donor and acceptor fluorophores as being optimal. For a particular single-stranded oligonucleotide, a skilled person would be in a position to select, within this range, the optimal distance for a specific first and second dye (donor and acceptor fluorophores) pair depending on their nature and character, the specific sequence of the target binding sequence and the RERS.

12. Appellant II's objection is not substantiated by verifiable facts as required by the established case law of the Boards of Appeal (cf. "Case Law", *supra*, II.C.6.1.4, page 318, *inter alia*, T 19/90, OJ EPO, 1990, page 476). Thus, the Main Request fulfils the requirements of Article 83 EPC.

Article 100(a) EPC; Article 54 EPC;
Claims 7-8

13. Claim 7 is a product-claim directed to a single-stranded oligonucleotide characterized by three structural features, namely a) a target binding sequence, b) a RERS located 5' to the target sequence, and c) a first and a second dye 8-20 nucleotides apart and at positions flanking the RERS. The FAM/DABCYL-labeled primer shown in Figure 24D of document D9, belonging to the state of the art according to Article 54(3) EPC, has been cited by appellant II as anticipating the subject-matter of claim 7 (cf. point XI *supra*).

13.1 It has to be resolved whether the oligonucleotide shown in Figure 24D of document D9 is a single-stranded oligonucleotide (cf. page 9, lines 17-22 and Figure 24D of document D9). As shown in Figure 24D, this oligonucleotide primer has a hairpin structure with a single-stranded loop and a double-stranded stem-loop. Appellant II argues that the presence of a hairpin structure in the oligonucleotide primer requires certain conditions (temperature, salt concentration, etc.) which are not part of the product itself. If these specific conditions are not present, then the oligonucleotide primer is completely, i.e. in its full-length, single-stranded (cf. point XI *supra*).

The high (thermodynamic) stability of hairpin structures is well-known in the technical field and acknowledged in document D9 itself (cf. page 29, paragraph [0203] and page 30, paragraph [0210] of document D9). For at least one of the primers shown in Figure 24 of document D9, in which the length of the 3' single-stranded primer sequence was reduced from 12 nucleotides (in the primer of Figure 24D) to 6 nucleotides (Figure 24G), "*the hairpin structure is the preferred conformation of this oligonucleotide, even at the 60°C annealing temperature*". This structure renders the primer useless for the amplification/detection methods disclosed in document D9 because "*the nucleotides in the stem and the loop of the hairpin are not available for hybridization to the target DNA*" (cf. page 31, paragraph [0214] of document D9).

In view thereof, the board considers the hairpin structure of the oligonucleotide primer of Figure 24D of document D9 to be maintained under standard, normal conditions, while only specific non-standard

conditions, requiring a purposive selection, would result in the oligonucleotide primer not having a hairpin structure.

13.2 Although not identified as such in document D9, the presence of the *AfaI/RsaI* RERS (5'-GTAC) within the sequence of the oligonucleotide primer of Figure 24D has not been contested by appellant I. However, under standard, normal conditions, in which this oligonucleotide primer has a hairpin structure, only part of this RERS is single-stranded (5'-TAC, as part of the hairpin loop) but not all of the RERS as required by feature b) of claim 7.

It is worth noting that the structural feature b) of claim 7 also contains a functional requirement, namely "*wherein all the restriction endonuclease site remains single stranded upon hybridization of the oligonucleotide to the target sequence*" (underlining by the board) (cf. point VIII *supra*). As shown in step 1 of Figure 10 of document D9, upon hybridization of the oligonucleotide primer to the target sequence, the primer maintains the hairpin structure and thus, not all of the RERS is single-stranded. In step 2 of Figure 10, the oligonucleotide primer has been fully linearized but it is double-stranded by complete hybridization to the target sequence. Thus, in none of these steps, the oligonucleotide primer disclosed in document D9 fulfils the requirement of claim 7(b).

13.3 Likewise, part (c) of claim 7 also contains a further functional requirement, namely that the defined structure of the dye pair is "*such that fluorescence of the first and second dye is quenched*" (cf. point VIII *supra*). Although this requirement is fulfilled by the oligonucleotide primer of Figure 24D of document D9, it

is questionable whether the required quenching is achieved in the presence of 20 nucleotides between the two dyes, or whether it is only achieved by the close proximity of both dyes resulting from the specific hairpin structure of the oligonucleotide primer. Indeed, as also shown in Figure 10 of document D9, upon hybridization of the primer to the target sequence and upon linearization of the primer and the creation of a double-stranded sequence, the quenching effect disappears and fluorescence is detected (cf. Figure 10 of document D19).

14. Thus, document D9 does not anticipate the subject-matter of claim 7 and the Main Request fulfils the requirements of Article 54 EPC.

Article 100(a) EPC; Article 56 EPC;
Closest prior art

15. Document D1, representing the closest prior art, discloses the adaptation and use of a detector probe in the strand displacement amplification method (SDA), a method already known in the prior art for amplification of a target DNA sequence (cf. page 400, Figure 1 of document D1). The detector probe is a single-stranded 5'-^{32}P labelled oligonucleotide which contains a HincII RERS site at its 5'-end and a target binding sequence at its 3'-end. In document D1, the disclosed method is referred to as the SDA/filtration system and it is further explicitly stated that this system is "*easily adaptable to a closed format using non-radiometric detection (e.g. fluorescence)*" (cf. page 399, right-hand column, lines 7-10 and page 403, right-hand column, second paragraph of document D1).

Objective technical problem and the solution proposed by the claimed subject-matter

16. In view of the explicit reference in document D1 to the adaptation of the SDA/filtration system to a closed format using a non-radiometric detection, in particular a fluorescence detection, the board does not consider the objective technical problem to be the provision of an alternative real-time detection method in general. Starting from document D1, the objective technical problem to be solved is considered to be the adaptation and use of a non-radiometric detector probe, in particular a fluorescence detector probe, for the SDA method/system of document D1 in a closed format. No hindsight is involved in the formulation of this problem since it is explicitly suggested in document D1 itself. In the light of the description, in particular of Examples 1 and 2, the board is convinced that this problem has been solved by the methods according to claims 1 and 3.

Obviousness and reasonable expectation of success

17. Appellant II has argued that the combination of the disclosures of document D1 and D2 renders the claimed subject-matter obvious (cf. point XI *supra*).

Document D2 refers to the relevance and advantages of real-time monitoring of nucleic acid amplification (PCR) reactions and to suitable instruments and systems for said monitoring, including known methods based on fluorescence energy transfer (FET) and the use of probes containing fluorescent-quenched pairs (cf. column 1, lines 10-62). Document D2 discloses a method for real-time detection of nucleic acid amplification using a self-quenching fluorescence oligonucleotide

probe (cf. column 3, line 5 to column 4, line 5). Indeed, the method relies on the extension of a primer annealed to the target polynucleotide with a DNA polymerase with 5'-3' exonuclease activity such that the oligonucleotide probe is degraded by this activity as it extends the primer and thus, as more and more probe is digested during amplification, a stronger and stronger fluorescent signal is generated (cf. *inter alia*, Figure 1, column 2, lines 5-12, column 3, lines 42-46).

18. In the board's view, however, the reference to fluorescence detection in document D1 would not have led a skilled person to the disclosure of document D2, let alone in an obvious manner.

18.1 A straightforward alternative derivable from this reference in document D1 would have been a mere replacement of the (^{32}P) radiometric label by a non-radiometric label from all non-radiometric labels available and known from the prior art, such as chemiluminescent labels, chromophore labels, ligands (such as biotin), fluorescence labels, etc.

18.2 Even if a skilled person, in the light of this reference in document D1, would have selected a fluorescence detection probe, there were also fluorescence probes available in the prior art which were different from the detection probes relying on a fluorophore-quenched pair and fluorescence energy transfer (FET), such as the fluorescence probes used for fluorescence polarization cited in the patent and developed by the authors of the patent-in-suit (cf. page 3, paragraph [0006] of the patent).

18.3 Also in the case a skilled person would have been aware of the prior art related to fluorescence detection probes used in the FET process, this prior art would not necessarily have led him/her in a straightforward manner to a fluorescence detection probe with the specific structure and properties of the single-stranded oligonucleotide (fluorescence) probe defined in claim 7. There were other possible alternatives available, such as the hairpin or double imperfect hairpin probes mentioned in the patent (cf. page 2, paragraph [0003] of the patent) or the probes shown in Figure 24 of document D9, none of them having the structure and properties of the claimed fluorescence probes.

19. It has also been argued by appellant II, that the reference in document D1 to a detection system described for the polymerase chain reaction (PCR) in which "*a detector probe is cleaved during extension of an upstream primer by a polymerase possessing a replication-dependent 5'-3' nuclease activity*" (cf. page 403, right-hand column, last paragraph of document D1), would have led a skilled person to document D2 in a straightforward manner (cf. point XI *supra*). The board, however, is not convinced by appellant II's argument.

19.1 The properties of this PCR detection system and of the detector probe used therein are neither disclosed in nor derivable from document D1, which only refers to a bibliographic reference (Holland et al., 1991. PNAS, Vol. 88, page 7276 to 7280), also cited in document D2 as describing the "*Background*" prior art referred to as the "*Tac-Man*" approach (cf. column 1, lines 33-39 and 53-62 of document D2). Neither the document Holland *et*

al. nor any other document disclosing the "*Tac-Man*" approach are on file.

19.2 From the bibliographic reference in document D1, it cannot be excluded that the document Holland *et al.* (*supra*) actually discloses a mere replacement of the radiometric label by a fluorescence label having the known advantages of these non-radiometric labels, as suggested in document D1 itself (cf. point 18.1 *supra*). As for the specific "*Tac-Man*" approach mentioned in document D2, there is no reference at all to such approach in document D1.

19.3 There are certainly cases in which the combination of two documents is made obvious by a third document which directly links these two documents. However, in the present case, the mere reference to a document, which, moreover, is not on file, precludes the board from accurately assessing its disclosure and deciding with certainty whether it actually links documents D1 and D2, as argued by appellant II, or whether the content of this document is only speculative and does not lead to a combination of documents D1 and D2, as argued by appellant I. As for experimental evidence, which, according to the established case law (cf. "Case Law", *supra*, I.D.10.9, page 231 and III.G.4.2.2.b, page 596), is not relevant if is not complete and sufficient for the board to examine it in detail and accuracy so as to arrive at a reliable decision, also the content of a document which is not on file and cannot be examined by the board, is considered not to be relevant.

19.4 In view of the above comments, the board is of the opinion that the reference in document D1 to a detection system described for PCR would not have led a skilled person to document D2.

20. Even if a skilled person would nevertheless have combined documents D1 and D2, in view of the probes disclosed in these two documents, substantial changes had to be carried out for arriving at the single-stranded oligonucleotide probe defined in claim 7. This would have required the combination of different features and the selection of suitable properties disclosed in these two documents (which could have been done only with hindsight knowledge of the patent).

20.1 On the one hand, as seen in points 18 and 19 above, the modification of the probe disclosed in document D1 would not be been straightforward and obvious. On the other hand, in view of the detection method disclosed in document D2 which is based on the necessary presence a DNA polymerase with a 5'-3' exonuclease activity (cf. point 17 *supra*), the introduction of a RERS between the two acceptor and donor dyes of the fluorophore-quenched pair probe would have been completely useless and, the presence of a restriction endonuclease, would render the method, if at all, more complex. In addition, the preferred blockage of the 3'-end of the probe disclosed in document D2 would render the probe useless in the SDA method disclosed in document D1 for which the DNA polymerase cannot have any 5'-3' exonuclease activity (cf. column 5, lines 38-40 of document D2).

20.2 Moreover, whilst for the radiometric probe used in the SDA method disclosed in document D1, there is no structural limitation linked to the the RERS within the probe, this is not the case for the probe used in the method disclosed in document D2, for which the acceptor and donor fluorophore dyes must be at a distance close enough for them to be quenched. References in document D2 to the known prior art identifies this distance to

be at about 6-16 nucleotides (cf. column 2, lines 45-54 of document D2).

20.3 However, the introduction of a RERS between the two fluorophore dyes of the probe disclosed in document D2 results in an additional structural limitation or steric requirement which is not present or taken into consideration in (any of the probes of) documents D1 or D2. As argued by appellant I (cf. point X *supra*), the distance or separation between the two fluorophore dyes must be short enough for the fluorophore pair to be quenched and long enough for the restriction endonuclease to be in a structurally appropriate configuration so as to be active and able to cleave the introduced RERS. Whereas for the first requirement ample information is provided in document D2 (*supra*), there is no guidance at all in document D2, let alone in document D1, for the second requirement.

20.4 It is the patent which identifies the distance range of 8-20 nucleotides, in appellant I's terms a window of opportunity, for which both, quenching between the acceptor and donor fluorophore dyes and cleavage of the RERS introduced between the two fluorophore dyes, is demonstrated. Nothing in the prior art documents on file suggests the existence of such a window of opportunity and, accordingly, there was no reason for a skilled person to expect its presence. Although, according to the established case law, the same level of skill has to be applied when, for the same invention, the two questions of sufficient disclosure and inventive step are considered, the two starting points differ, since for inventive step (Article 56 EPC) the skilled person knows only the prior art, whereas for sufficiency of disclosure (Article 83 EPC), the skilled person not only knows this prior art but

also the disclosure of the invention. In the present case, this additional knowledge would essentially be the presence of the window of opportunity (cf. points 11 and 12 *supra*).

21. Thus, the Main Request fulfils the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent on the basis of the new Main Request filed at the oral proceedings on 20 October 2014 and the description to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated