BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:
(A) [ ] Publication in OJ
(B) [ ] To Chairmen and Members
(C) [X] To Chairmen
(D) [ ] No distribution

Datasheet for the decision of 26 January 2012

Case Number: T 1263/09 - 3.3.08

Application Number: 06011667.0

Publication Number: 1736554

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions

Applicant:
CORNELL RESEARCH FOUNDATION, INC.

Headword:
Coupled PCR/CORNELL

Relevant legal provisions:
EPC Art. 123(2), 54, 56
EPC R. 139

Keyword:
"Main request - obvious error and correction (yes)"
"Added subject-matter (no)"
"Novelty and inventive step (yes)"

Decisions cited:
-

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1263/09 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 26 January 2012

Appellant: (Applicant)
CORNELL RESEARCH FOUNDATION, INC.
20 Thornwood Drive
Suite 105
Ithaca
NY 14850 (US)

Representative:
Miles, John Stephen
Potter Clarkson LLP
Park View House
58 The Ropewalk
Nottingham NG1 5DD (GB)

Decision under appeal: **Decision of the Examining Division of the European Patent Office posted on 8 January 2009 refusing European patent application No. 06011667.0 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: M. Wieser
Members: P. Julià
R. Moufang

Summary of Facts and Submissions

I. European patent application No. 06011667.0 (published as EP 1 736 554, hereinafter "*the application as filed*"), a divisional application of the earlier European patent application No. 97927787.8 (published as International patent application WO 97/45559), was refused by the examining division.

II. The application as filed disclosed three different embodiments, namely i) a process comprising a primary polymerase chain reaction (PCR) followed by a secondary PCR and a ligase detection reaction (LDR) (PCR/PCR/LDR process), ii) a LDR/PCR process, and iii) a PCR/PCR process. Claim 1 of the application as filed was directed to the third embodiment and read as follows:

"1. A method for identifying two or more of a plurality of sequences differing by one or more single-base changes, insertions, deletions, or translocations in a plurality of target nucleotide sequences comprising:

providing a sample potentially containing one or more target nucleotide sequences with a plurality of sequence differences;

providing one or more primary oligonucleotide primer groups, each group comprised of one or more primary oligonucleotide primer sets, each set **characterized by** (a) a first oligonucleotide primer, having a target-specific portion and a 5' upstream secondary primer-specific portion, and (b) a second oligonucleotide primer, having a target-specific portion and a 5' upstream secondary primer-specific

portion, wherein the first oligonucleotide primers of each set in the same group contain the same 5' upstream secondary primer-specific portion and the second oligonucleotide primers of each set in the same group contain the same 5' upstream secondary primer-specific portion, wherein the oligonucleotide primers in a particular set are suitable for hybridization on complementary strands of a corresponding target nucleotide sequence to permit formation of a polymerase chain reaction product, but have a mismatch which interferes with formation of such a polymerase chain reaction product when hybridized to any other nucleotide sequence present in the sample, and wherein the polymerase chain reaction products in a particular set may be distinguished from other polymerase chain reaction products in the same group or other groups;

providing a polymerase;

blending the sample, the primary oligonucleotide primers, and the polymerase to form a primary polymerase chain reaction mixture;

subjecting the primary polymerase chain reaction mixture to two or more polymerase chain reaction cycles comprising a denaturation treatment, wherein hybridized nucleic acid sequences are separated, a hybridization treatment, wherein the target-specific portion of the primary oligonucleotide primers hybridize to the target nucleotide sequences, and an extension treatment, wherein the hybridized primary oligonucleotide primers are extended to form primary extension products complementary to the target nucleotide sequence to which the primary oligonucleotide primer is hybridized;

providing one or a plurality of secondary oligonucleotide primer sets, each set **characterized by** (a) a first secondary primer, having a detectable reporter label and containing the same sequence as the 5' upstream portion of a first primary oligonucleotide primer, and (b) a second secondary primer containing the same sequence as the 5' upstream portion of a second primary oligonucleotide primer from the same primary oligonucleotide primer set as the first primary oligonucleotide *complementary to* the first secondary primer, wherein a set of secondary oligonucleotide primers amplify the primary extension products in a given group;

blending the primary extension products, the secondary oligonucleotide primers, and the polymerase to form a secondary polymerase chain reaction mixture;

subjecting the secondary polymerase chain reaction mixture to two or more polymerase chain reaction cycles comprising a denaturation treatment, wherein hybridized nucleic acid sequences are separated, a hybridization treatment, wherein the secondary oligonucleotide primers hybridize to the primary extension products, an extension treatment, wherein the hybridized secondary oligonucleotide primers are extended to form secondary extension products complementary to the primary extension product; and

detecting the labeled secondary extension products, thereby indicating the presence of one or more target nucleotide sequences in the sample." (in cursive and underlined by the board).

Claim 2 to 4 were directed to specific embodiments of claim 1. Claims 5 to 26 related to the first embodiment, i.e. the PCR/PCR/LDR process.

III. The examining division considered that none of the requests on file at the oral proceedings before it, namely a Main Request filed on 18 October 2007, Auxiliary Requests 1 to 5 filed on 11 November 2008 and Auxiliary Request 6 filed on 28 November 2008, fulfilled the requirements of the EPC. The Main Request and Auxiliary Requests 1, 3 and 4 were considered to contravene Article 82 EPC, Auxiliary Requests 2 and 5 not to meet the requirements of Article 123(2) EPC and Auxiliary Request 6 to contravene Article 56 EPC. This Auxiliary Request 6, consisting of four claims, was the sole request directed to a single embodiment, namely to the PCR/PCR process. Claim 1 of this Auxiliary Request read as follows:

"1. A method for identifying two or more of a plurality of sequences differing by one or more single-base changes, insertions, deletions, or translocations in a plurality of target nucleotide sequences comprising:

providing a sample potentially containing two or more of a plurality of sequences differing by one or more single-base changes, insertions, deletions or translocations in a plurality of target nucleotide sequences; ...

[as in claim 1 of the application as filed]

... detecting the labeled secondary extension products, thereby indicating the presence of two or more of a plurality of sequences differing by one or more single-base changes, insertions, deletions or translocations in a plurality of target nucleotide sequences."

Claims 2 to 4 were directed to embodiments of claim 1.

IV. The applicant (appellant) filed a notice of appeal and a statement setting out its grounds of appeal together with a Main Request and Auxiliary Requests 1 to 8. The Main Request and Auxiliary Requests 1 to 6 were identical to the requests underlying the decision under appeal, whereas Auxiliary Requests 7 and 8 were new claim requests. The appellant further requested a refund of the appeal fee pursuant to Rule 103(1)(a) EPC on the grounds that substantial procedural violations were committed during the examination proceedings. As a precautionary measure, oral proceedings were also requested (Article 116 EPC).

V. The examining division did not rectify its decision and referred the case to the Boards of Appeal (Article 109(2) EPC).

VI. On 14 October 2011, with the summons to oral proceedings, the board sent a communication pursuant to Article 15(1) RPBA informing the appellant of its preliminary, non-binding opinion on substantive matters, in particular those concerning Articles 82 and 84 EPC. In this communication, two new documents were introduced into the proceedings (documents D3 and D4; cf. Section X *infra*).

VII. On 28 December 2011, the appellant replied to the board's communication and filed a new Main Request and new Auxiliary Requests 1 to 5 with replacement pages of an adapted description. With a letter dated 3 January 2012, a retyped version of the replacement pages of the description was provided.

VIII. Oral proceedings were held on 26 January 2012. At these proceedings, the appellant withdrew its previous Main Request and filed a Replacement Main Request which was also withdrawn during the proceedings and, at 12.25 am, replaced by a New Replacement Main Request. The request for reimbursement of the appeal fee was also withdrawn at the end of the oral proceedings.

IX. Appellant's New Replacement **Main Request** contained two claims. Claim 1 read as follows:

"1. A method for identifying two or more of a plurality of sequences differing by one or more insertions or deletions in a plurality of target nucleotide sequences comprising:

providing a sample potentially containing two or more of a plurality of sequences differing by one or more insertions or deletions in a plurality of target nucleotide sequences;

providing one or more primary oligonucleotide primer groups, each group comprised of more than one primary oligonucleotide primer sets, each set characterized by (a) a first oligonucleotide primer ...

[as in claim 1 of the application as filed]

... and an extension treatment, wherein the hybridized primary oligonucleotide primers are extended to form primary extension products complementary to each of the two or more of a plurality of sequences differing by one or more insertions or deletions in the plurality of target nucleotide sequences to which the primary oligonucleotide primer is hybridized;

providing one or a plurality of secondary oligonucleotide primer sets, each set characterized by (a) a first secondary primer, having a detectable reporter label and containing the same sequence as the 5' upstream portion of a first primary oligonucleotide primer, and (b) a second secondary primer containing the same sequence as the 5' upstream portion of a second primary oligonucleotide primer from the same primary oligonucleotide primer set as the first primary oligonucleotide *primer sequence contained by* the first secondary primer, wherein a set of secondary oligonucleotide primers amplify the primary extension products in a given group; ...

[as in claim 1 of the application as filed]

... an extension treatment, wherein the hybridized secondary oligonucleotide primers are extended to form secondary extension products complementary to each of the primary extension products; wherein the polymerase chain reaction secondary oligonucleotide primers in a particular set produce secondary extension products of unique length so that they may be distinguished from

other nucleic acids in the secondary polymerase chain reaction mixture, said method further comprising:

separating the extension products by size or electrophoretic mobility and distinguishing the secondary extension products which differ in size, and

detecting the labeled secondary extension products, thereby indicating the presence of two or more of a plurality of sequences differing by one or more insertions or deletions in a plurality of target nucleotide sequences." (in cursive and underlined by the board).

Claim 2 was identical to claim 3 of the application as filed and directed to an embodiment of claim 1.

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

D1: US 5 512 441 (publication date: 30 April 1996);

D2: WO 96/15271 (publication date: 23 May 1996);

D3: Z. Lin et al., Proc. Natl. Acad. Sci. USA, March 1996, Vol. 93, pages 2582 to 2587;

D4: P. Belgrader et al., Genome Science & Technology, 1996, Vol. 1, No. 2, pages 77 to 87.

XI. The submissions of the appellant, insofar as they are relevant to the present decision, may be summarized as follows:

Rule 139 EPC and Article 123(2) EPC

The correction of claim 1, namely the replacement of the term "*complementary to*" by "*primer sequence contained by*" (cf. Sections II and IX *supra*), removed only an obvious inconsistency present in that claim. It was clear from the part of claim 1 preceding this correction that the first secondary primer contained the same sequence as the 5' upstream portion of the first primary oligonucleotide primer. The sequence of the first primary oligonucleotide primer was not - and could not be - complementary to that of the first secondary primer. This was also clear from Figure 23 which illustrated the PCR/PCR process of claim 1 and in which it was clear that the first primary oligonucleotide primer and the first secondary oligonucleotide primer shared the same "zip code" sequence, so that the first secondary oligonucleotide primer could hybridize to the PCR-synthesised complement of the first primary oligonucleotide primer. This was further supported by the explanation of step 2 of the method shown in Figure 22 found on page 20, lines 36 to 38, paragraph [0112] of the application as filed.

The correction of claim 1 was also obvious in the sense that nothing else could have been considered by the skilled person because the use of secondary oligonucleotide primers with sequences complementary to those of the primary oligonucleotide primers was technically meaningless. Likewise, it was also meaningless to use a first secondary oligonucleotide primer having the same sequence as that of the first primary oligonucleotide primer and a second secondary

oligonucleotide primer having a complementary sequence to that of the second primary oligonucleotide primer. The explanation given for step 2 of the PCR/PCR process shown in Figure 23 on page 20, lines 49 to 51, paragraph [0113] of the application as filed was plainly erroneous and incorrect. Thus, the requirements of Rule 139 EPC were fulfilled.

Moreover, the correction of claim 1 did not introduce new subject-matter and all amendments made in that claim had a formal basis in the application as filed.

Article 54 EPC

Document D1 did not mention insertions or deletions, it disclosed only a method of detecting a point mutation in a nucleic acid sequence. This method involved the selective amplification of a mutant gene sequence using a first PCR amplification of both mutant and wild-type sequences, a first restriction enzyme digestion of only the wild-type sequence and a second PCR amplification of undigested amplified fragments of the mutant sequence only. The coupled PCR process of claim 1 required a first and second PCR amplification of both the mutant and the wild-type sequences of a plurality of target nucleotide sequences. The coupling of a first and second PCR was not considered in document D2 which disclosed a multiplex amplification method in which the first step was an oligonucleotide ligation assay (OLA). Document D3 described a multiplex PCR amplification method, wherein three PCR rounds were involved. However, none of these PCR rounds used primary and secondary oligonucleotide primer sets having the features required by claim 1. Moreover, there was no teaching to

add a detectable reporter label to the secondary extension products as a means of indicating the presence of two or more of a plurality of sequences differing by one or more insertions or deletions in a plurality of target sequences. Likewise, there was no teaching in document D4 for identifying insertions or deletions in a plurality of target nucleotide sequences since this document was concerned only with the detection of single nucleotide variants.

Article 56 EPC

The method disclosed in document D1 was inherently unsuited for identifying two or more of a plurality of sequences differing by one or more insertions or deletions in a plurality of target nucleotide sequences because that method was based on detecting a single product with a single base mismatch. In order to arrive at the method of claim 1, the skilled person had to redesign, in the absence of any motivation thereto, the whole concept of the method disclosed in document D1. None of the methods taught in the other documents of the cited prior art could readily be combined with the method of document D1 and, even if the skilled person were to attempt this combination, it would not have arrived at the claimed method.

Likewise, there was nothing in document D2 that could have led the skilled person to arrive at the method of claim 1. The OLA step disclosed in document D2 was not interchangeable with the PCR step of claim 1 because the former was used to achieve allelic discrimination of a single base change and not to amplify a target nucleotide sequence. Neither document D2 nor any other

of the prior art documents on file provided any suggestion or reason to replace the OLA step by a PCR step or any other amplification method, particularly when any apparent need for amplification was achieved - in the process of document D2 - by the PCR step taking place after the OLA step.

The three PCR rounds of the multiplex PCR amplification method disclosed in document D3 allowed a nested primer approach in which two primers that hybridized to the same strand of the target polynucleotide were used in subsequent rounds of amplification. The use of nested primers was described as enhancing amplification specificity. Since it was stated that a multiplex PCR with a large number of primers and thus, an increased primer sequence complexity, could result in unpredictable nonspecific amplification, the skilled person had no incentive to remove the nested primers and to change the design of the approach disclosed in that document.

Document D4 disclosed a method for typing single base variations and there was no motivation to consider this method as a starting point for arriving at the method of claim 1. None of the other documents on file could overcome the deficiencies of this document.

XII. The appellant (applicant) requested that the decision under appeal be set aside and that a patent be granted on the basis of claims 1 and 2 of the New Replacement Main Request (12:25).

Reasons for the Decision

New Replacement Main Request
Rule 139 EPC

1. The appellant has requested a correction under Rule 139 EPC in the definition of the secondary oligonucleotide primer sets of claim 1 - for comparison see the text, in cursive and underlined by the board, in claim 1 of the application as filed and in claim 1 of the New Replacement Main Request (cf. Sections II and IX *supra*, respectively).

2. In the application as filed, each set of the secondary oligonucleotide primer sets is characterized by (a) a first secondary primer which is required to contain the same sequence as the 5' upstream portion of a first primary oligonucleotide primer, and (b) a second secondary primer which is also required to contain the same sequence as the 5' upstream portion of a second primary oligonucleotide primer. Although no ambiguity arises from these definitions, it arises immediately thereafter when defining the second primary oligonucleotide primer as being from the same primary oligonucleotide primer set as the first primary oligonucleotide "*complementary to*" the first secondary primer (cf. Section II *supra*). Thus, confusion arises as to whether the second secondary primer has the same sequence as the second primary primer, as stated in the first part of the definition, or whether it is complementary to that sequence, as seems to be implied by the definition of the primer set to which it belongs. Therefore, the board agrees with the appellant that the

presence of an error in the wording of claim 1 was immediately obvious to the skilled person.

3. The use of a first secondary primer having the same sequence as that of the first primary primer with a second secondary primer having a sequence complementary to that of the second primary primer is technically meaningless. It is known that, for a PCR amplification, the oligonucleotide primers must be suitable for hybridization on complementary strands of the corresponding target nucleotide sequence - as required in the definition of the primary PCR amplification in claim 1 (cf. Section II *supra*). Thus, both primers used in the secondary PCR amplification must have the same sequences as those of the corresponding primers used in the primary PCR amplification (such as indicated on page 20, lines 36 to 38, paragraph [0112] of the application as filed which describes the PCR/PCR process shown in Figure 22), or they must both have complementary sequences to those of the corresponding primers used in the primary PCR amplification (such as indicated on page 20, lines 49 to 51, paragraph [0113] of the application as filed which describes the PCR/PCR process shown in Figure 23).

4. The appellant argues that, only and exclusively, the former alternative would be technically meaningful for a skilled person since the latter alternative, in spite of the statement made in paragraph [0113] of the application as filed, namely "*primers complementary to the 5' ends of the primary PCR primers ... are then used to amplify the primary PCR extension products ...*" (cf. page 20, lines 49 to 51, paragraph [0113] of the application as filed), does not permit the formation of

PCR products. A PCR amplification can only take place in a 5' to 3' direction. Indeed, according to the appellant, the statement made in that paragraph is also an obvious error immediately evident to the skilled person because no PCR amplification could take place with the complementary PCR primers. It is also noted that in claim 5 of the application as filed, which was directed to the first embodiment disclosed in the application, i.e. the primary PCR/secondary PCR/LDR process, the secondary oligonucleotide PCR primers and PCR primer sets were defined as proposed now by the appellant in amended claim 1.

5. There is no reason for the board not to believe appellant's assertion and therefore, the board considers the amendment of claim 1 as the obvious and sole possible meaningful correction of an error immediately evident to the skilled person. The requirements of Rule 139 EPC are thus fulfilled.

Article 123(2) EPC

6. The subject-matter of claim 1 has a basis in paragraph [0112] of the application as filed which describes the PCR/PCR process shown in Figure 22. The subject-matter of this claim is illustrated in both Figures 22 and 23 of the application as filed. Indeed, formal basis is found in the technical teaching of the application as a whole and the common general knowledge of the skilled person.

7. The requirement that the hybridized secondary oligonucleotide primers in the secondary PCR are extended to form secondary extension products

complementary to "*each of*" the primary extension products, and the corresponding requirement in the primary PCR for the extension of the hybridized primary oligonucleotide primers to "*each of*" the (plurality of the) target sequences, has a formal basis in Figures 22 and 23 which require the amplification of "*all*" primary products using zip-code primers. It is noted that this requirement is also found in all the figures of the application as filed that illustrate embodiments of the application with a coupled primary PCR/secondary PCR (see also page 5, line 40 and page 6, line 54 to 55 of the application as filed for the PCR/PCR/LDR process).

8. The other amendments introduced into claim 1 ("*more than one*"), in particular the introduction of the subject-matter of claim 2 of the application as filed, have a formal basis in the original claims and in the description of the application as filed.

9. No objections were raised by the examining division under Article 123(2) EPC in the decision under appeal and, in the light of the considerations made above, the board does not see any reason to deviate from this finding.

Article 84 EPC

10. The examining division did not raise any objection under Article 84 EPC against any of the examined claim requests. The board does not see any reason to do so now of its own motion.

Article 54 EPC

11. The board considers that none of the documents of the cited prior art discloses a method for identifying two or more of a plurality of sequences differing by one or more insertions or deletions in a plurality of target nucleotide sequences using a coupled PCR, i.e. a primary and a secondary PCR amplification, with (first and second) primary and (first and second) secondary oligonucleotide primers - and primer sets - as required in claim 1, and wherein each of the primary extension products is extended to form secondary extension products of unique length so that they are separated by size or electrophoretic mobility, distinguished by their different size and detected by the presence of a detectable reporter label.

12. Indeed, no objection for lack of novelty in the light of the disclosure in documents D1 or D2 was raised in the decision under appeal for any of the examined claim requests. The board sees no reason to deviate from this view. The same applies to the teaching of document D3 (*infra*), which is based on three coupled PCR reactions, wherein, in principle, none is carried out using simultaneously a first and second oligonucleotide primer having a target-specific portion and a 5' upstream secondary primer-specific portion or, as named in document D3, a 5' upstream universal primer (tail) (cf. page 2582, right-hand column, 3rd to 5th paragraphs and Figure 1 of document D3). Document D3 does not disclose a separation of the (secondary or final) extension products by size or electrophoretic mobility, distinguishing these products which differ in size, and their detection by the presence of a label, since an

essential step of the method disclosed in document D3 is a restriction enzyme digestion of the extension products (cf. page 2584, right-hand column, 4th paragraph and Figure 1 of document D3).

13. In a communication pursuant to Article 15(1) RPBA (cf. Section VI *supra*), the board introduced documents D3 and D4 into the appeal proceedings and noted that some of the authors of document D4 are named as inventors of the application as filed. The board also explicitly referred to the fact that it was unaware of the exact date of publication of document D4 and that it was not known therefore whether or not this document was citable under Article 54(2) EPC. Since there is no further information on file in this regard, the board refrains from any further reference to document D4 in this decision.

14. Thus, the board considers that the requirements of Article 54 EPC are fulfilled.

Article 56 EPC

15. The method of document D1 comprises two PCR amplifications in which the first PCR is carried out using upstream and downstream "long-tail" primers. "Long-tail" primers are defined as comprising a complementary portion which is complementary to one of the nucleic strands in the (target) genomic duplex and a non-complementary portion which is not complementary to either of the strands in the duplex (cf. *inter alia* column 7, lines 51 to 58 of document D1). These primers are "*synthesized or selected so as to mediate a restriction site in a specific codon of a synthesized*

strand if and only if the codon is present in the strand with the wild-type nucleotide sequence" (cf. *inter alia* column 8, lines 23 to 27 of document D1). An essential step of this method is the treatment of the duplexes synthesized in the first PCR amplification with a specific restriction enzyme in a digestion step in which "normal" (wild-type) nucleotide sequences will be cleaved but not "mutant" nucleotide sequences (cf. *inter alia* column 8, lines 27 to 35 and 50 to 62, Figure 2 of document D1).

This essential (digestion) step is not part of claim 1 which, contrary to the method disclosed in document D1, requires all primary extension products to be amplified in the second PCR amplification. Moreover, there is no reference in document D1 to use the disclosed method for identifying one or more insertions or deletions in a target sequence. In view of these different technical features between the method of claim 1 and that disclosed in document D1, the board considers that document D1 does not, and cannot, represent the closest prior art.

16. The method disclosed in document D2 allows the detection of point mutations and to discriminate alleles differing by a single base (cf. *inter alia* page 10, lines 34 to 38 of document D2). There is no reference to use this method for identifying one or more insertions or deletions in a target sequence. As starting material, the target sequence must be in a single stranded form in order to carry out the ligation of the - continuous (Figure 1) or two distinct (Figure 3) - split probe reagents (SPRs) which are complementary to and hybridized specifically with the

target sequence (cf. *inter alia* page 12, lines 1 to 26 of document D2). The SPRs further comprise 5' upstream non-complementary regions (NCR). In the embodiment shown in Figure 3, the first NCR has a sequence that is complementary to that of a first amplification primer and the second NCR has a sequence that is identical to the sequence of a second primer which is itself complementary to the 3' end sequence of the extension product of the first primer (cf. *inter alia* page 12, lines 27 to page 13, lines 14, Figures 1a-b, 3a-b of document D2). Once a ligated probe is formed, the next step involves a PCR amplification using the probe as template. The first primer hybridizes to its complementary region within the first NCR of the SPR and is extended, wherein, as stated above, the 3' end sequence of the extension product includes a site complementary to the sequence of the second primer (cf. Figures 1c-d, 3c-d), thereby permitting the exponential PCR amplification (cf. *inter alia* page 14, lines 6 to 28, Figures 1e-f, 3e-f of document D2).

Contrary to the opinion of the examining division, the board considers that neither the substitution of the first (ligation) step for a PCR amplification nor the selection of a specific embodiment of document D2 (using two distinct SPR, Figure 3) nor the change of the target sequence from a single stranded form to a double stranded form and, accordingly, the modification of the primers design, can be seen as obvious to the skilled person in the absence of a pointer in this direction or without the hindsight knowledge of the disclosure of the present application. Thus, document D2 is considered not to represent the closest prior art

for assessing the claimed invention in the light of the problem-solution approach.

17. It is the disclosure of document D3 which, in the board's view, represents the closest prior art. This document discloses a multiplex PCR method based on three coupled PCR amplifications, wherein, in the first PCR round, a locus-specific primer (O) and a first hybrid primer (L) - consisting of a 5' universal primer sequence portion (tail 1, T1) and a 3' locus-specific primer portion - are used and, in the second PCR round, a single universal primer identical to the sequence of T1 and a second hybrid primer (P) - consisting of a 5' universal primer sequence portion (tail 2, T2) and a 3' locus-specific primer portion - are used, wherein, in order to enhance the amplification specificity, the primers O and P are nested so that the O primer is outside with respect to the P primer (cf. page 2582, right-hand column 4th paragraph, page 2585, right-hand column, 1^{rst} paragraph of document D3). In the third PCR round, the first and second primers have sequences identical to T1 and T2, respectively (cf. page 2582, right-hand column, 3rd to 5th paragraphs and Figure 1 of document D3). The use of group-specific tails is also contemplated in document D3 (cf. page 2583, paragraph bridging left and right-hand columns of document D3).

18. The presence of nonspecific PCR products is explicitly addressed in document D3, which describes a clean-up step after the first PCR round and, as rightly pointed out by the appellant (cf. Section XI *supra*), the advantageous use of the nested O and P primers for enhancing the PCR specificity and efficiency (cf. page 2585, left-hand column, 2nd paragraph and

right-hand column, 1st paragraph of document D3). However, it is also stated that, for primers generating nonspecific products with T1, it is helpful to replace T1 with the corresponding L primers in the second PCR round (cf. page 2586, left-hand column, last paragraph of document D3). In that case, both (L and P) primers used in the second PCR round contain a 5' universal primer sequence portion (a 5' upstream secondary primer-specific portion) and a 3' locus-specific sequence portion (a target-specific portion) being thus similar to those used in the primary PCR mixture of claim 1, whereas the first and second primers (T1 and T2) used in the third PCR round are similar to those used in the secondary PCR mixture of claim 1. According to document D3, these PCR conditions have been used for amplifying five loci, including locus F7, a polymorphic site which is a 10-bp insertion/deletion (cf. page 2584, left-hand column, 2nd paragraph and page 2586, paragraph bridging left and right-hand columns of document D3).

19. Starting from this closest prior art, the technical problem to be solved may be seen in the provision of an alternative method for identifying two or more of a plurality of sequences differing by one or more insertions or deletions in a plurality of target nucleotide sequences. As a solution to this problem, the application proposes the method according to claim 1. The board is convinced that the technical problem is solved.

20. Contrary to the method disclosed in document D3, the method according to claim 1 does not contemplate a restriction enzyme digestion for analysing the final extension PCR products (cf. page 2584, right-hand

column, 4th paragraph and Figure 1 of document D3). Although document D3 refers to the fact that, when designing primers, the lengths of the final PCR products need to be taken into consideration, this is done only for separating, identifying and detecting these products in the context of the allelic products generated by a restriction enzyme digestion (cf. page 2583, right-hand column, 2nd paragraph to page 2584, 1st paragraph of document D3). In the light of the whole content of the disclosure of document D3, either when taken alone or in combination with any other prior art document on file, the board considers that the substitution of the restriction enzyme digestion step described in the method of document D3 for the separation, identification and detection steps required in the method of claim 1 of the New Replacement Main Request would not have been obvious to the skilled person in the absence of an indication thereto or without the hindsight knowledge of the disclosure of the application.

21. Thus, the board considers that the requirements of Article 56 EPC are fulfilled.

Article 83 EPC

22. No objections were raised under this article by the examining division in the decision under appeal nor does the board see any reason to raise any of its own.

Adaptation of the description

23. In view of the amendments introduced into the New Replacement Main Request and the amendments required to be introduced into the description in order to be in line with the correction made under Rule 139 EPC, the board decides to remit the case to the first instance for adapting the description.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to grant a patent on the basis of claims 1 and 2 of the New Replacement Main Request (12:25) and a description and figures yet to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski M. Wieser