DECISION
of 19 January 2006

Case Number: T 0707/03 - 3.3.04
Application Number: 98123569.0
Publication Number: 0930370
IPC: C12Q 1/68
Language of the proceedings: EN
Title of invention:
Labeled primer for use in detection of target nucleic acids
Applicant:
ZLB Behring GmbH
Headword:
Mismatch primer/ZLB BEHRING
Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 123(2)
Keyword:
"Added subject-matter (no)"
"Clarity, conciseness, support, sufficiency of disclosure, novelty, inventive step (yes)"
Decisions cited:
-
Catchword:
-
DECISION
of the Technical Board of Appeal 3.3.04
of 19 January 2006

Appellant: ZLB Behring GmbH
Emil-von-Behring-Strasse 76
D-35041 Marburg (DE)

Decision under appeal: Decision of the Examining Division of the European Patent Office posted 17 February 2003 refusing European application No. 98123569.0 pursuant to Article 97(1) EPC.

Composition of the Board:
Chair: U. Kinkeldey
Members: G. Alt
         G. Weiss
Summary of Facts and Submissions

I. The appeal was lodged against the decision of the Examining Division to refuse the European patent application No. 98 123 569.0 with the title "Labeled primer for use in detection of target nucleic acids" pursuant to Article 97(1) EPC.

II. Claims 1, 4, 7, 8, 10 and 12 as originally filed read:

"1. A process for the detection of a target nucleic acid in a sample comprising single-stranded DNA said process comprising

(a) contacting said sample with a forward and/or reverse primer,

wherein at least one primer carries a label or part of a label system in a 3' terminal deliberately mismatched portion of said primer relative to the target nucleic acid, said mismatched portion amounting to at least one nucleotide, preferably 2 to 5 or more nucleotides,

to create a mixture of duplexes during hybridization conditions with said forward and/or said reverse primer annealed to complementary DNA sequences each of said target nucleic acid in case of its presence;

(b) maintaining the mixture of step (a) with a template-dependent nucleic acid polymerase having a 3' to 5' proofreading activity or a mixture of enzymes having such proofreading activity under conditions sufficient to permit the 3' to 5' nuclease activity of said polymerase or mixture of enzymes to cleave the
annealed primer in its 3' mismatched portion thereby releasing label or part of a label system;

(c) detecting and/or measuring the release of label or part of a label system.

4. A process according to one of the preceding claims, wherein the label is a reporter-quencher molecule pair linked to the 3' and 5' terminal regions of the forward and/or the reverse primer.

7. A process according to one of the preceding claims wherein the said nucleic acid polymerase or said mixture of enzymes are thermostable.

8. A process according to one of the preceding claims wherein a multiplicity of targets are detected simultaneously by suitable selection of primers and labels respective label systems.

10. A kit for the detection of a target nucleic acid in a sample comprising labelled primers used in the processes of claims 1, 2, 3, 4, 5, 6, 7 or 8 and a suitable nucleic acid polymerase or mixture of enzymes.

12. A reaction mixture for detecting a target nucleic acid which reaction mixture comprises prior to amplification labeled primers used in the processes of claims 1, 2, 3, 4, 5, 6, 7 or 8 and a suitable nucleic acid polymerase or mixture of enzymes."

III. The Examining Division referring to documents WO-A-90 12115 (D1), WO-A-97 29210 (D2) and US 5,804,375 (D3) decided that claims 1, 4, 7, 8 and 12 of the only
set of claims before them (corresponding to the set of claims as originally filed) did not meet the requirements of Article 54 EPC in view of document D1 disclosing a process wherein label was removed by a 3' to 5' nuclease activity from an oligonucleotide in case of a mismatch in the base pairing between a nucleotide in the oligonucleotide and a DNA and the free label subsequently detected. Moreover, they decided that the subject-matter of claims 1 to 12 did not involve an inventive step in the light of the disclosure of either of document D2 or D3 in combination with the common general knowledge. The process disclosed in documents D2 and D3 only differed from the claimed one in that a nucleic acid polymerase having a 5' to 3' nuclease activity was employed to remove the label at the 5' end of a primer. Since enzymes with 3' to 5' nuclease activity were known, it was straightforward to use them in combination with a mismatched oligonucleotide to carry out the methods disclosed in documents D2 or D3. Finally, claims 8, 10 and 12 were held not to comply with the requirement of clarity according to Article 84 EPC because in claims 10 and 12 the primers were undefined; moreover, some claim references in said claims were wrong. Claim 8 contained the unclear term "suitable selection of primers and labels respective label systems".

IV. With the statement of grounds of appeal a new set of claims was submitted containing amended claims 8 and 10. In the context of arguing inventive step the appellant noted that document D3 was published after the earliest priority date. He introduced document US 5,210,015 (hereinafter referred to as document D4) which, in the context of the assessment of inventive step, he
considered as containing a teaching equivalent to that of document D3.

V. After having summoned for oral proceedings, the Board issued a communication setting out its preliminary views on some of the issues, for example, that document D1 seemed to disclose subject-matter falling under the terms of claims 1 and 12.

VI. In response a new main request containing amended claims was submitted.

VII. During the oral proceedings a further new main request was filed in which claim 1 was amended, a new claim 3 was added, claims 2-12 were renumbered as claims 3 to 12, the numbering was adapted in claims (new numbering) 4, 7, 11 and 12 and former claim 12 was deleted. Overall, the request contained 10 claims to a process (independent claim 1 and dependent claims 2 to 10), and 2 claims to a kit (independent claim 11 and dependent claim 12).

VIII. Claims 1, 3, 9 and 11 of the main request filed during oral proceedings read:

"1. A process for the detection of a target nucleic acid in a sample comprising single-stranded DNA said process comprising

(a) contacting said sample with a forward and/or reverse primer,

wherein at least one primer carries a label or part of a label system in a 3' terminal portion of the primer
and wherein said primer is selected such that at least one, preferably at least the last two to five or more nucleotides at the 3' end of the primer are not complementary to the nucleic acid sequence to be detected

to create a mixture of duplexes during hybridization conditions with the said forward and/or said reverse primer annealed to complementary DNA sequences each of the target nucleic acid in case of its presence;

(b) maintaining the mixture of step (a) with a template-dependent nucleic acid polymerase having a 3' to 5' proofreading activity or a mixture of enzymes having such proofreading activity under conditions sufficient to permit the 3' to 5' nuclease activity of said polymerase or mixture of enzymes to cleave off the 3' mismatched portion of the annealed primer thereby releasing label or part of a label system;

(c) detecting and/or measuring the release of label or part of a label system.

3. A process according to claim 2 in which the release of the label is directly proportional to the amount of amplified DNA.

9. A process according to one of the preceding claims wherein a multiplicity of nucleic acid targets is detected simultaneously by selection of corresponding primers wherein at least one primer for each target carries a label or part of a label system in the 3' terminal portion the primer.
11. A kit for the detection of a target nucleic acid in a sample comprising labeled primers used in the processes of claims 1 to 10 and a suitable nucleic acid polymerase having a 3' to 5' proofreading activity or a mixture of enzymes having such proofreading activity.

IX. The arguments submitted by the appellant in writing and during oral proceedings as far as they are relevant to the present decision can be summarized as follows:

Article 123(2) EPC

The amended passage in claim 1 was based on claim 1 as originally filed. New claim 3 was supported by page 8, lines 13 to 15 as originally filed. Basis for claim 9 was found in claims 1 and 8 as originally filed and for claim 11 in claim 1 and 10 as originally filed.

Article 84 EPC

Claims 8 and 10 objected to by the Examining Division for lack of clarity (renumbered claims 9 and 11) were now clear, because in claim 9 the primer and in claim 11 the nucleic acid polymerase were precisely defined.

Article 54 EPC

Claims 1 to 10

Document D1 disclosed a process for detecting the presence or absence of a nucleotide at a specific location wherein detection was accomplished by an oligonucleotide with a sequence completely
complementary with the target DNA sequence. In contrast, the application related to a process for detecting nucleic acid fragments, like for example viral DNA, and used a primer which was mismatched with respect to the target DNA.

Claims 11 and 12

The claimed kits differed from those disclosed in document D1 by the relationship between the primer and the target DNA.

Inventive step

Claims 1 to 12

Document D3 was the closest prior art document. In contrast to the detection process disclosed therein the claimed process needed a reduced number of primers and a polymerase with 3' to 5' nuclease activity. Thus, the problem to be solved was a simplification of the process described in document D3. The solution of the problem in the claimed way was not obvious because the processes disclosed in documents D2 and D3 exclusively relied on 5' to 3' nuclease activity. The mismatch situation with subsequent cleavage of the mismatch by a 3' to 5' nuclease activity occurring during the process of document D1 would not have been taken as a suggestion to modify the process disclosed in document D3 because the process in document D1 served a completely different purpose.
X. Request

The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of claims 1 to 12 filed during oral proceedings.

Reasons for the Decision

Article 123(2) EPC

1. The following passages in the application documents as originally filed are a basis for the amendments filed during appeal proceedings:

   - The amended passage in claim 1 "and wherein said primer is selected such that at least one, preferably at least the last two to five or more nucleotides at the 3' end of the primer are not complementary to the nucleic acid sequence to be detected" (emphasis added by the Board) is based on claim 1 as originally filed reading: "A process for the detection of a target nucleic acid in a sample comprising a single-stranded DNA said process comprising (a) contacting a sample with a forward and/or reverse primer, wherein at least one primer carries a label or part of a label system in a 3' [...] mismatched portion of said primer [...] said mismatched protein amounting to at least one nucleotide, preferably 2 to 5 or more nucleotides, .... " (emphasis added by the Board). The term "mismatched" in claim 1 as originally filed is regarded as synonymous to the expression "are not complementary to the nucleic acid sequence".

1280.D
Claim 3 reading "A process according to claim 2 in which the release of the label is directly proportional to the amount of amplified DNA" (emphasis added by the Board) is based on page 8, lines 12 to 15: "In addition to this the method which is described here is very well suited for quantitative amplification, since the increase in fluorescence is directly proportional to the quantity of amplified DNA." (emphasis added by the Board). The generalisation of the term "fluorescence" to "label" is supported by the application documents as originally filed as a whole because the skilled person takes from them that the application of the process is not technically linked to the type of the label.

Claim 9 is based on claim 1, part (a) as originally filed "wherein at least one primer carries a label or part of a label system in a 3' terminal, deliberately mismatched portion of said primer" and claim 8 as originally filed relating to the simultaneous detection of multiple targets.

Claim 11 is based on claim 1(b) as originally filed "maintaining the mixture of step (a) with a template-dependent nucleic acid polymerase having a 3' to 5' proofreading activity or a mixture of enzymes having such proofreading activity" and claim 10 as originally filed "A kit for the detection of a target nucleic acid in a sample comprising labelled primers used in the processes of claims [...] and a suitable nucleic acid polymerase or mixture of enzymes.".
The further amendments in the claims concern their renumbering and adaptation of references to claims. The amendments in the description which concern the replacement of the word "relates" on page 2 ("The invention therefore relates ...") by the word "uses" and the removal of the text of the former claims form page 20 do also not add matter.

Hence, the claims of the main request fulfil the requirements of Article 123(2) EPC.

Article 84 EPC

2. The lack of clarity due to inadequate definition of primers objected to by the Examining Division with regard to previous claim 8 is removed since claim 9 now specifies that the primer corresponds to the target sequence. Previous claim 10 (now claim 11) is clarified by defining the polymerase as in claim 1 as a "... suitable nucleic acid polymerase having a 3' to 5' proofreading activity or a mixture of enzymes having such activity...". Thus, the Board considers amended claims 9 and 11 as clear and does not see other objections under Article 84 EPC.

Hence the claims fulfil the requirements of Article 84 EPC.

Article 83 EPC

3. No objections were raised during the examination proceedings or in the decision under appeal under
Article 83 EPC. The Board has no reason to doubt the sufficiency of the disclosure of the claimed invention.

Hence the claims fulfil the requirements of Article 83 EPC.

**Article 54 EPC**

**Claims 1 to 10**

4. According to the process of claim 1 nucleic acid is detected by an oligonucleotide which is labelled at its 3' end and wherein at least one nucleotide at this 3' end is non-complementary with the nucleotide(s) at this position in the sequence to be detected. In case of the formation of a hybrid, i.e. if the nucleic acid to be detected is present in the sample, there is no base pairing at the 3' end. The protruding, labelled nucleotides are removed by a 3' to 5' nuclease activity and the label can be detected.

5. Document D1 discloses a process for determining the existence or non-existence of a particular nucleotide at a specific location on a strand of nucleic acid. Although it is not explicitly mentioned in the document, it may be considered to implicitly disclose that this process can be used for the same purpose as the claimed one, namely to detect complete nucleic acid fragments. The features of the process are as follows: A nucleic acid fragment is exposed to an oligonucleotide complementary to a locus of interest within that fragment and carrying a label at a nucleotide at or near the position of a suspected variant nucleotide. The oligonucleotide and the nucleic acid are allowed to
hybridize which either creates a match or a mismatch at the position of the nucleotide to be determined. The oligonucleotide-nucleic acid-duplex is then treated with a nucleolytic activity, which generally is a 3' to 5' nuclease (page 13, lines 20-22). In case of a match of the nucleotides at the position in question the label is retained at the oligonucleotide. In case of a mismatch the nuclease removes the mismatched nucleotide(s) and thus also the label which may be separated and determined (page 31, last paragraph continued on page 32).

6. It is true that both, the claimed process and that disclosed in document D1 encompass the removal of mismatched bases from a hybridized oligonucleotide by a 3' to 5' nuclease activity. However, they differ in the structure of the oligonucleotide to hybridise to the target nucleic acid, a feature characterized in part (a) of claim 1. Whereas in the claimed process the sequence is non-complementary at the 3' end with a given target sequence, it is designed to be completely complementary with the target sequence according to the process disclosed in document D1. Hence, in practice, if the same target sequence were to be detected by either of the processes, the sequences of the oligonucleotides for hybridization were different depending on the process.

Consequently, the subject-matter of claim 1 and dependent claims 2 to 10 is novel.

Claims 11 and 12
7. Claim 11 relates to a kit comprising, inter alia, "labeled primers". They are defined by the functional feature "used in the processes of claims 1 to 10". This definition includes by virtue of its reference to the process of claims 1 to 10 the structural features by which the oligonucleotides of claim 1 are characterized. Hence, the primers of claim 1 and those of claim 11 are defined by the same structural features. Therefore, the reasons leading to the finding of novelty of the claimed process over that disclosed in document D1 apply as well to the kits of claim 11 and 12 which are therefore novel over the kits disclosed in the same document.

The subject-matter of claims 1 to 12 fulfills the requirements of Article 54 EPC.

Inventive step

Claims 1 to 12

8. Document D3, a US patent, has a publication date between the third priority and the filing date of the present application. It was referred to by the Examining Division in the evaluation of inventive step. However, in the examination file the Board could not detect a sign that an examination of the validity of the priority has taken place in order to justify that the document fulfills the requirements of Article 54(2) EPC and can thus be used for the evaluation of inventive step.

In the appeal proceedings the appellant has introduced document US 5,210,015 (herein referred to as document 1280.D
D4) published on 11 May 1993, i.e. before the first priority date and therefore a document pursuant to Article 54(2) EPC. As apparent from the first paragraph of document D3, document D3 is a continuation of a US patent claiming priority of a an International application which is a continuation-in-part of document D4. The appellant has implicitly acknowledged ("Das Gleiche gilt, mutatis mutandis für D3 (als US 5,210,015;...)") and the Board agrees that the disclosures of documents D3 and D4 which are relevant for the evaluation of inventive step, are equivalent. Therefore, the assessment of inventive step will be carried out in view of the disclosure of document D4.

9. In proceedings before the European Patent Office, the problem-solution-approach is generally applied to assess inventive step. It involves as a first step the identification of the closest prior art document. The closest prior art document discloses subject-matter which is conceived for the same purpose or aiming at the same objective as the claimed invention and which has the most technical features in common with it. Therefore, at first it has to be determined which of documents D1, D2 and D4 represents the closest prior art document.

10. The subject-matter of claim 1 relates to a process for the detection of a target nucleic acid in a sample. It is essential for the detection that the label or part of the label system is released (step (b) and (c) of claim 1) so that it can subsequently be detected. In its most general form, the subject-matter of claim 1 is a process for the detection of nucleic acid independent of nucleic acid amplification.
11. According to the process disclosed in document D1, which, as stated in point 5 above, may implicitly be considered as suitable for the same purpose as the claimed process, the label is released from the oligonucleotide only in case of a mismatch after hybridization, but not in case of a match. In contrast, according to the process disclosed in document D4, the label is always cleaved from the oligonucleotide after hybridization has occurred. Therefore, this process comes closer to the claimed one as that disclosed in document D1.

12. Document D2, which was also considered as a possible closest prior art document in the decision under appeal discloses a process which can be used concurrent with nucleic acid amplification, whereas in document D4, both, a nucleic acid amplification (polymerization)-dependent and a nucleic acid amplification (polymerization)-independent detection process are disclosed.

Since the most general embodiment of claim 1 is a process that is not linked to nucleic acid amplification, the so-called polymerization-independent process of document D4 (column 2, lines 27-47; column 6, lines 1 and 2) is regarded as the closest piece of prior art.

13. The features of this process are as follows: A sample comprising single-stranded nucleic acid is contacted with i) a non-labelled oligonucleotide having a sequence complementary to a region of the target nucleic acid and ii) a labelled oligonucleotide having
a sequence complementary to a second region of the target nucleic acid. The sequence of the oligonucleotides is designed such that they anneal in close proximity, i.e. the 3' end of the first, unlabelled oligonucleotide is adjacent to the 5' end of the labelled oligonucleotide. To this mixture a template-dependent nucleic acid polymerase having a 5' to 3' nuclease activity is added. The reason for annealing two oligonucleotides is that cleavage occurs more efficiently, if the 3' end of an upstream oligonucleotide provides the initial binding site for the nuclease activity (column 6, lines 48-56). If target nucleic acid is present in a sample, both, the unlabelled and the labelled oligonucleotide anneal to it and the 5' to 3' nuclease activity cleaves the labelled oligonucleotide, thus releasing labelled nucleotides or nucleic acid fragments which can be detected after having been separated.

14. In view of the closest prior art document the Board sees the problem underlying the claimed process in the provision of an alternative process for the detection of the presence of a target nucleic acid in a sample.

15. This problem is solved by the claimed process having the following essential elements which distinguish it from the process of the closest prior art document: i) an oligonucleotide labelled at the 3' end and non-complementary at the 3' end with the nucleic acid sequence to be detected and ii) a template-dependent nucleic acid polymerase having 3 to 5' proofreading (or in other words "nuclease") activity.
In Examples 1 and 2 Hepatitis C RNA and Hepatitis B DNA, respectively, is detected according to the claimed process. Hence, the Board considers that the problem is indeed solved by the subject-matter as claimed.

16. For the assessment whether the solution to the above formulated problem as provided in the claims involves an inventive step, it has to be considered whether the skilled person starting from document D4 was led in an obvious manner by either document D4 or other prior art documents on file or by the common general knowledge to solve the above formulated problem by choosing the claimed combination of features.

17. Document D4 is silent about enzymes with a 3' to 5' nucleolytic activity.

18. Nevertheless, enzymes having 3' to 5' nuclease or proofreading activity were known before the first priority date of the application. In their natural surroundings such an activity is useful to detect and avoid wrong base pairings during reproduction of nucleic acid. The Board considers that in view of this knowledge a skilled person seeking an application for such an enzymatic activity would envisage applying it in a similar context, namely to discriminate between matched and mismatched base pairings. The teaching of document D1, the only prior art document relating to the 3' to 5' nuclease activity, supports this view because in the process disclosed therein the capability of a 3' to 5' nucleolytic activity to detect and remove mis-pairings is exploited for determining the existence or non-existence of a nucleotide in a nucleic acid.
19. However, the aspect of discrimination between "right" or "wrong" base pairings has nothing to do with the claimed process where, in contrast, a mismatch is deliberately introduced in order to trace the presence or absence of a nucleic acid fragment. Consequently, given the difference in the concepts between the two processes, in the Board's view, a skilled person would not be led in an obvious manner either by the common general knowledge or by the teaching of document D1 to modify the process of document D4 such as to arrive at the claimed process.

20. This reasoning also applies to the kits of claims 11 and 12 since they are characterized by the inventive combination of a mismatched labelled primer and a nucleic acid polymerase having 3' to 5' nuclease activity.

Hence, the subject-matter of claims 1 to 12 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 department of first instance with the order to grant a patent on the basis of:
claims 1 to 12 filed during oral proceedings
- pages 2 and 20 of the description as filed during oral proceedings
- pages 3 to 19 of the description as published
- Figure 1 as published.

Registrar: P. Cremona

Chair: U. Kinkeldey