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D E C I S I O N
of 25 February 2005

Case Number: T 1093/02 - 3.3.4

Application Number: 87310363.4

Publication Number: 0272009

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Nucleic acid probes for detection and/or quantitation of non-viral organisms

Patentee:
Gen-Probe Incorporated

Opponent:
-

Headword:
Nucleic acid probes/GEN-PROBE INC.

Relevant legal provisions:
EPC Art. 123(2), 123(3), 83, 87-89, 54, 56

Keyword:
"Added subject-matter (no)"
"Right to priority, sufficiency of disclosure, novelty, inventive step (yes)"

Decisions cited:
G 0002/98

Catchword:
-

Case Number: T 1093/02 - 3.3.4

D E C I S I O N

of the Technical Board of Appeal 3.3.4
of 25 February 2005

Appellant: (Proprietor of the patent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 7 August 2002 concerning maintenance of European patent No. 0272009 in amended form.**

Composition of the Board:

Chairwoman: U. Kinkeldey
Members: M. Wieser
R. Moufang

Summary of Facts and Submissions

I. The appeal was lodged by the Patent Proprietors (Appellants) against the decision of the Opposition Division, whereby the European Patent No. 0 272 009 was maintained in amended form according to Article 102(3) EPC. The patent claims priority from US 934244 (24 November 1986) and US 83542 (7 August 1987).

The patent had been granted on the basis of a set of claims 1 to 329 for all designated contracting states except ES and a different set of claims 1 to 329 for ES.

II. The patent had been opposed by one party under Article 100(a) EPC on the grounds of lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC).

III. The Opposition Division had decided that the main request and auxiliary requests 1 to 5 before them contravened the requirements of Article 123(2) EPC, but that claims 1 to 150 for all designated contracting states according to auxiliary request 6 met the requirements of the EPC.

IV. The Opponents, who had responded to the Appellants' grounds for appeal with a letter of 4 July 2003, informed the Board on 9 February 2005 that they withdrew their opposition.

V. The Board had issued a communication on 3 September 2004. Oral proceedings were held on 25 February 2005.

The Appellants requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request filed at the oral proceedings and a description amended thereto.

VI. The new main request for all designated states consisted of claims 1 to 169. Claims 1, 30 and 34 thereof read as follows:

"1. A method for preparing a probe for use in a qualitative or quantitative hybridization assay which comprises constructing an oligonucleotide that is sufficiently complementary to hybridize under hybridization conditions to an rRNA sequence selected to be unique to a non-viral organism or group of non-viral organisms sought to be detected, said oligonucleotide being selected by:

a) identifying one or more candidate variable regions by comparing one or more variable region rRNA sequences from a non-viral target organism or group of non-viral target organisms with one or more variable region rRNA sequences from the known nearest related organism to select a sequence unique to the rRNA of the target organism or organisms; and

b) synthesizing an oligonucleotide complementary to the unique rRNA sequence, wherein the thermal stability of probe:target nucleic acid hybrids is greater than the thermal stability of probe:nontarget nucleic acid hybrids;
wherein said target organism or organisms are selected from one or more species in a genus or one or more genera in a family.

30. A method for preparing a probe or combination of probes for use in a qualitative or quantitative hybridization assay which comprises constructing a nucleotide polymer that is sufficiently complementary to hybridize to a region of rDNA or rRNA selected to distinguish a target non-viral organism or first group of non-viral organisms sought to be detected from at least one nontarget organism or second group of nontarget organisms which may be present in a sample, wherein said nontarget organisms or group of organisms are close phylogenetic relatives of said target organisms or group of organisms, said region of rDNA or rRNA being selected by:

- comparing one or more rDNA or rRNA nucleotide base sequences of said non-viral organism or group of non-viral organisms sought to be detected with one or more rDNA or rRNA nucleotide base sequences of said nontarget organisms or group of nontarget organisms;

- aligning said rDNA or rRNA nucleotide base sequences of said non-viral organism or group of non-viral organisms with said rDNA or rRNA nucleotide base sequences of said nontarget organisms or group of organisms so as to identify regions of homology; and

- selecting said nucleotide polymer by maximizing the homology of said nucleotide polymer to the regions of said rDNA or rRNA of said non-viral organism or non-viral group of organisms sought to be detected while minimizing the homology of said

nucleotide polymer to rDNA or rRNA sequences of said nontarget organism or group of organisms sought to be distinguished therefrom; wherein said organism or group of organisms sought to be detected is selected from one or more species in a genus or one or more genera in a family.

34. A method for preparing a probe for use in a qualitative or quantitative hybridization assay which comprises constructing an oligonucleotide that is sufficiently complementary to hybridize to a region of rDNA or rRNA selected to be unique to a non-viral organism or group of non-viral organisms sought to be detected, said region of rDNA or rRNA being selected by:

- comparing one or more rDNA or rRNA nucleotide base sequences of said non-viral organism or group of non-viral organisms sought to be detected with one or more rDNA or rRNA nucleotide base sequences of its closest phylogenetic relatives;

- aligning said rDNA or rRNA nucleotide base sequences of said non-viral organism or group of non-viral organisms with said rDNA or rRNA sequences of said closest phylogenetic relatives, so as to reveal the interspecies hypervariable rDNA or rRNA regions;

- selecting said probe oligonucleotide in said interspecies hypervariable region by maximizing the homology of said probe oligonucleotide to the regions of said rDNA or rRNA of said non-viral organism or non-viral group of organisms sought to

be detected while minimizing the homology of said probe oligonucleotide to rDNA or rRNA sequences of said closest phylogenetic relatives sought to be distinguished therefrom; wherein said organism or group of organisms sought to be detected is selected from one or more species in a genus or one or more genera in a family."

Dependent claims 2 to 29, 31 to 33 and 35 to 37 referred to preferred embodiments of the claimed methods.

Claims 38 to 169 corresponded to claims 11 to 126 and 135 to 150 of the patent as maintained by the Opposition Division in the decision under appeal. Several of the claims contain back-references to a method according to claims 1 to 37. In so far as the claims refer to products they have been adapted only with respect to the back-references contained therein.

VII. The present decision refers to the following documents:

(1) Annual Meeting of the American Society for Microbiology, Abstract G14, March 1985

(2) Annual Meeting of the American Society for Microbiology, Abstract C-90, March 1986

(3) Sixth International Symposium on Human Chlamydial Infections, Sanderstead, Surrey, 20-21 June 1986, pages 88 to 92

(4) EP-A-0 232 085

(5) EP-A-0 245 129

(6) WO 84/02 721

VIII. The submissions made by the Appellants as far as they are relevant to the present decision may be summarised as follows:

The claims were entitled to the second priority date, namely 7 August 1987; US 83542, with the result that documents (4) and (5) were state of the art according to Article 54(3) EPC. The technical features contained in the characterising parts of claims 1, 30 and 34 were not disclosed in the prior art documents on file. These claims were therefore novel (Article 54 EPC). The closest prior art document (6) disclosed a method, based on differential nucleic acid hybridization, for the preparation of a mixed probe specific for a target organism. The problem underlying the present invention according to claims 1 to 37 was to provide an improved method for this purpose. The claimed solution could not have been arrived at in an obvious way, neither from the disclosure in document (6) alone, nor in combination with any of documents (1) to (3). Claims 1 to 37 involved an inventive step (Article 56 EPC).

IX. The submissions made by the Opponents in their letter of 4 July 2003 as far as they are relevant to the present decision may be summarised as follows:

Appellants should not be permitted to defend claims in appeal proceedings which exceed the scope of the main request at issue in opposition proceedings.

Claim 1 violated Articles 123(2) and 123(3) EPC. All claims not contained in the set of claims as maintained by the Opposition Division (claims 1 to 37) were not entitled to any priority date and lacked novelty and inventive step (Articles 54 and 56 EPC) in the light of prior art documents (1) to (6). Since these claims lacked any technical feature, a skilled person was not able to carry out the invention without undue burden, contrary to the requirements of Article 83 EPC.

Reasons for the Decision

1. Claims 1 to 37 of Appellants' present main request are broader in scope than the claims of their main request before the Opposition Division.

   In a situation where the patent is maintained in amended form and the Patent Proprietors are the Appellants, they may in appeal proceedings pursue claims which are broader than those held to be allowable by the Opposition Division. The Appellants (Patent Proprietors) are entitled to revert to a more broadly worded version of the patent, and in particular the one as granted, even if they have filed a restricted version during the opposition proceedings or at the commencement of appeal proceedings (cf Case Law of the Boards of Appeal of the EPO, 4th Ed. 2001, VI.I.3.1.2 (b), pages 348 to 349, English version).

   Thus, an argument against the introduction of such broader claims in appeal proceedings must fail.

2. The patent has been granted on the basis of two different sets of claims, one for all designated contracting states except ES and another one for ES. The two sets of claims were identical except for the order of the claims.

Maintenance of the patent on the basis of one set of claims for all designated contracting states does not, therefore, violate the requirements of Article 123(3) EPC.

Claims 1 to 37

Added subject-matter - Articles 123(2) and 123(3) EPC

3. Article 100(c) EPC, added subject-matter (Article 123(2) EPC), has not been raised as ground of opposition.

Claim 1 results from a combination of claims 1 and 2 as granted. The protection conferred by the claim has been reduced with regard to claim 1 as granted. The same applies to claim 30 resulting from a combination of claims 31 and 32 as granted. Claims 2 to 29 correspond to claims 3 to 30 as granted. Claims 31 to 37 correspond to claims 33 to 39 as granted.

Thus, claims 1 to 37 do not violate the requirements of Articles 123(2) and 123 (3) EPC.

Sufficiency of disclosure - Article 83 EPC

4. This issue was not a ground for opposition but was examined by the Opposition Division on its own motion in accordance with Article 114(1) EPC (cf point (23) of the decision under appeal).

The Board has therefore to examine if the requirements of Article 83 EPC are met, in particular if the claimed invention can be carried out by a skilled person without undue burden.

5. In step (a) of the characterising part of claim 1 the skilled reader is instructed, in order to identify and select a sequence unique to the rRNA of a target organism, to compare rRNA sequences of the target organism with rRNA sequences from the known nearest related organism. Step (b) instructs the reader to synthesize an oligonucleotide probe complementary to the unique rRNA sequence, identified and selected in step (a), and to verify that the thermal stability of probe:target nucleic acid hybrids is greater than the thermal stability of probe:nontarget nucleic acid hybrids.

6. According to claims 30 and 34 a probe is prepared by first aligning rDNA or rRNA from a target organism with rDNA or rRNA from a nontarget organism, being a close phylogenetic relative (claim 30) or the closest phylogenetic relative (claim 34), to identify regions of homology. A nucleotide probe is then selected by maximizing the homology of the nucleotide to the rDNA or rRNA regions of the target organism while minimizing the homology of the nucleotide to the rDNA or rRNA regions of the nontarget organism.

7. The patent contains 21 examples wherein the claimed methods are carried out by applying the technical features described in claims 1, 30 and 34. The Board accepts that, compared to the plethora of embodiments

claimed, this may prima facie look to be a small number. However, the Board sees no evidence on file that this number of examples is not sufficient. Thus, no case has been made out that the patent in suit does not disclose the invention in a manner sufficiently clear and complete to be carried out by a skilled person without undue burden, as required by Article 83 EPC and the case law of the Boards of Appeal.

Priority - Articles 87 to 89 EPC

8. According to feature b) of claim 1, the thermal stability of probe:target nucleic acid hybrids is compared with the thermal stability of probe:nontarget nucleic acid hybrids and is found to be greater than the latter.

9. The second priority document, US 83542 (7 August 1987), defines on page 10, last paragraph, the melting temperature (Tm) of oligonucleotide/rRNA hybrids as being the temperature at which 50% of the probe is hybridized and 50% is single-stranded. In the passage bridging pages 11 to 12 it is said that the rate of hybridization will increase as ionic strength of the reaction mixture increases and that the thermal stability of hybrids will increase with increasing ionic strength.

It belongs to the common knowledge of a skilled person in the field of biotechnology that ionic strength increases with the degree of complementarity between a probe and a target nucleic acid.

The priority document contains nine examples which correspond to examples 1 to 7, 9 and 10 of the patent in suit. All these examples refer to a protocol of sequence alignment, as being the subject-matter of present claims 30 and 34, which is disclosed on page 5 of the priority document.

According to example 1, in the passage bridging pages 17 and 18 of the priority document, the partial rRNA sequences of the target organism and of its closest taxonomic neighbours are aligned for maximum nucleotide homology. From this alignment regions unique to the target organism are determined. A probe is selected which is perfectly complementary to a target rRNA and which has a mismatch with the rRNA from its known closest related taxonomic neighbour. The probe sequence is characterised by the criteria of length, Tm and sequence analysis.

10. The Board is convinced that a skilled person can derive the subject-matter of claims 1, 30 and 34 directly and unambiguously, using common general knowledge, from the previous application as a whole. Thus, the requirement for claiming priority of the "same invention" referred to in Article 87(1) EPC as defined by the Enlarged Board of Appeal in the decision G 2/98 (OJ 2001, 413) is met.

11. Therefore, documents (4) and (5), having a publication date lying after 7 August 1987, the filing date of the second priority document US 83542, belong to the state of the art according to Articles 54(3) and (4) EPC, and will not be considered for the question of inventive step (Article 56 EPC).

Novelty - Article 54 EPC

12. None of the prior art documents on file discloses a method having the characterising technical features of either of claims 1, 30 or 34.

In detail, none of these prior art documents teaches to compare rRNA sequences from a target organism with rRNA sequences from a nontarget organism, which is the known nearest related organism, in order to select a sequence unique to rRNA of the target organism, and to synthesize an oligonucleotide which forms hybrids with target nucleic acids which are thermally more stable than hybrids with nontarget nucleic acids (claim 1).

In the same way, the documents on file do not disclose to align rRNA or rDNA from a non-viral target organism with rRNA or rDNA of a nontarget organism thought to be distinguished therefrom, which nontarget organism is a close phylogenetic relative (claim 30) or its closest phylogenetic relative (claim 34), and to select a polymer by maximizing homology to target regions while minimizing homology to nontarget regions.

Therefore, the subject-matter of claims 1 to 37 is novel and meets the requirements of Article 54 EPC.

Inventive step - Article 56 EPC

13. Claims 1, 30 and 34 each refers to a method for preparing a probe for use in a qualitative or quantitative hybridization assay which probe is unique

to a non-viral target organism, or group of non-viral target organisms.

14. The closest prior art results from document (6), disclosing a method aiming at the same purpose.

This method is the subject of claim 23 of document (6) and relies on the principle of differential nucleic acid hybridization. rRNA from a target organism is used as a template to synthesize marked cDNA. The purified cDNA fragments (probes) are then fractionated by hybridizing them with excess of rRNA of nontarget organisms, which may be organisms evolutionarily most closely related to the target organism. The non-adsorbed fraction is collected, concentrated and again hybridized with nontarget rRNA. The non-adsorbed fraction obtained after several repetitions of these working steps is a preparation consisting of a mixed probe specifically hybridizing to the target organism (cf page 29, line 27 to page 30, line 9; example 1, pages 61 to 65; example 3, pages 80 to 81; pages 92 to 93).

15. In the passage bridging pages 42 and 43 (under the heading "Procedure C") document (6) refers to a different approach for the production of specific rRNA probes which does not rely on differential nucleic acid hybridization. Based on the fact that nucleotide sequences of **rRNA from widely different organisms** have been determined, it is stated that group specific sequences similar to a specific group of organisms can be identified by **comparing these known sequences**. A sequence complementary to this group specific sequence can then be chemically synthesized and marked.

Thus, instead of comparing rRNA sequences from a target organism with rRNA from a close, or the closest, phylogenetic relative, document (6) in "procedure C" suggests to compare total rRNA from widely different organisms.

Moreover, except from the short passage bridging pages 42 and 43, this procedure is not referred to at any other place in the description, examples or claims of document (6), which are concerned with differential nucleic acid hybridization methods only.

16. In the light of the disclosure in the closest prior art, the problem to be solved by the patent in suit is considered to be the provision of an improved method for preparing a probe for use in a qualitative or quantitative hybridization assay.

This problem has been solved by the methods disclosed in claims 1, 30 and 34.

17. The characterizing technical features of claims 1, 30 and 34 are not disclosed in document (6). Moreover, the document does not contain information that would encourage the skilled person to amend its disclosure and to arrive at the subject-matter of claims 1, 30, and 34 in an obvious way. These characterizing technical features are:

- comparison (alignment) of rRNA (or rDNA) sequences from a target organism with rRNA from the known nearest related organism (claim 1), or from a close (claim 30) or the closest (claim 34)

phylogenetic relative. (Document (6) on pages 42 to 43 generally mentions comparison of rRNA sequences from widely different organisms);

- synthesizing of oligonucleotide probes wherein the thermal stability of probe:target nucleic acid hybrids is greater than the thermal stability of probe:nontarget nucleic acid hybrids (claim 1);

- selecting a probe by maximizing its homology to rRNA or rDNA of the target organism while minimizing its homology to sequences of nontarget organisms (claims 30 and 34).

18. For the assessment of an inventive step according to the requirements of Article 56 EPC the Board has to examine if the disclosure in the remaining prior art documents on file, namely documents (1) to (3), would have prompted the skilled person to amend the methods disclosed in document (6) and to arrive at the subject-matter of claims 1, 30 and 34 in an obvious way.

19. Document (1), a short abstract, reports synthesizing of a 14-bases long oligonucleotide based on published Mycoplasma rRNA/rDNA. The probe, designated MYC 14, is said to be specific for Mycoplasma and not to show crosshybridization to genomic digests of eubacterial and eukaryotic DNA. The sequence complementary to MYC 14 has been detected at the 5'-end of the 16S rRNA of *Mycoplasma pneumoniae*.

20. Document (2), another abstract, describes the cloning of DNA sequences from *Campylobacter jejuni* in *E.coli*. The nucleotide sequence of the 16S rRNA of

Campylobacter has been compared to other, published, bacterial 16S rRNA sequences. It has been found that several regions of rRNA are specific for *Campylobacter.* Two oligonucleotide probes (AR 196 and AR 197) were synthesized based on these specific regions. The probes did not hybridize to *E.coli* rRNA, or to DNA from *Enterobacter, Pseudomonas* and *Vibrio.*

21. Document (3) discloses the identification of a DNA probe complementary to a rRNA sequence unique to specific strains of *Chlamydia trachomatis* (Trachoma and LGV Biovar), which allows to distinguish them from other closely related strains. The 16S rRNA of a target strain was purified, partially cleaved and labelled and used as probes in a Southern hybridization assay with DNA coding for 16S rRNA of target strains and a closely related strain. Different hybridization patterns were obtained. Based on these results a 31 base oligonucleotide probe with inverse complementarity to a unique region of the target strain 16S rRNA was synthesized. This probe was found to be unique for the target strains of *Chlamydia trachomatis* (see page 89, last paragraph to page 90).

Thus, the preparation of target organism specific rRNA probes in document (3) relies on differential nucleic acid hybridization methods as disclosed in document (6).

22. To summarise, none of prior art documents (1) to (3) contains information that would encourage the skilled reader to amend the method disclosed in the closest prior art document (6) by applying the working steps being the technical characterising features of the

methods of claim 1, 30 and 34, listed in point (17) above.

The subject-matter of these claims and of claims dependent thereon, involves an inventive step and meets the requirements of Article 56 EPC.

Claims 38 to 169

23. These claims refer to nucleotide polymers, nucleic acid hybrids and hybridisation assays and methods for preparing probes. Several of the dependent claims refer back to the methods of claims 1 to 37. Except for the back references to preceding claims they are corresponding to claims 11 to 126 and 135 to 150 of the patent as maintained by the Opposition Division in the decision under appeal.

These claims, as a result of the appeal filed by the Patent Proprietors, are subject of the appeal proceedings. No observations with regard to these claims have been submitted by the Opponents in their letter dated 8 July 2003.

The Board sees no reason to depart from the decision of the Opposition Division finding that these claims meet the requirements of the EPC (cf points (20) to (25) of the decision under appeal).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of

   - claims 1 to 169 (for all designated states) of the main request filed at the oral proceedings

   - amended pages 3 to 5 of the description filed at the oral proceedings and pages 2 and 6 to 66 (line 46) of the description as granted

   - figures 1 to 11 as granted.

The Registrar: The Chairwoman:

P. Cremona U. Kinkeldey