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D E C I S I O N
of 11 October 2001

Case Number: T 0822/98 - 3.3.4

Application Number: 87304433.3

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Title of invention:
Hybridisation probes

Patentee:
BIO-RAD LABORATORIES, INC.

Opponent:
Perkin Elmer Corp.

Headword:
Probes/BIO-RAD

Relevant legal provisions:
EPC Art. 54, 84, 111

Keyword:
"Main request and first auxiliary request - lack of clarity
(yes) - no clear distinction over the prior art"
"Second auxiliary request - remittal for further prosecution"

Decisions cited:
T 0047/90

Catchword:
-



Case Number: T 0822/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 11 october 2001

Appellant: BIO-RAD LABORATORIES, INC.
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Decision under appeal: Decision of the Opposition Division of the
European Patent Office posted 2 June 1998
revoking European patent No. 0 246 864 pursuant
to Article 102(1) EPC.

Composition of the Board:

Chairman: L. Galligani
Members: F. L. Davison-Brunel
V. Di Cerbo

Summary of Facts and Submissions

I. The appeal was lodged by the patent proprietors against the decision of the opposition division, dated 2 June 1998, whereby the European Patent No. 0 246 864 (Application No. 87304433.3; priority: 19 May 1986 GB 8612087), which had been opposed by one party on grounds of Article 100(a) EPC, namely lack of novelty and lack of inventive step, was revoked.

Basis of the decision were claims 1 to 11 as filed on 29 April 1998. The opposition division considered that, while the subject-matter of method claims 1 to 8 was novel, that of product claims 9 to 11 lacked novelty having regard to the following document:

- (1) EP-A-0 185 494, a European patent application published on 25 June 1986, claiming priority from US 681055 of 13 December 1984.

Independent claims 1 and 9 of the set of claims before the opposition division read as follows (in bold-type character the differences in comparison with the corresponding claims 1 and 11 as granted):

"1. A method for discriminating between alternative nucleotide sequences, which method comprises subjecting adjacent segments of a target base sequence to hybridisation with a detectable first nucleotide probe and with a second nucleotide probe, to form a hybrid, the nucleotide sequence of the first and second probe being such that where they form a split probe hybrid with a complementary target sequence they may subsequently be linked, subjecting any hybrid obtained to linkage, and detection of any hybrid obtained;

the DNA sequence of the detectable first nucleotide probe and of the second nucleotide probe being such that a potential mismatch in the target sequence lies either between the said probes or at the terminal end of one of said probes which is contiguous with the other of the said probes; the method being effected such that a complementary target sequence is discriminated from a target sequence with one or more non-complementary nucleotides **by means of the linkage step.**"

"9. A split probe hybrid **for use in the method of claim 1**, comprising a detectable first nucleotide probe and a second nucleotide probe hybridised to adjacent segments of a target base sequence, the detectable first nucleotide probe being capable of linkage to the second nucleotide probe, characterised in that the detectable first nucleotide probe and/or the second nucleotide probe are hybridised to either side of a variant sequence associated with a disease state or to the corresponding normal sequence; or are hybridised to the target base sequence such that a variant base sequence associated with a disease state therein is at the terminal end of one of said probes, which terminal end is contiguous with the other of said probes or are hybridised to the corresponding normal sequence."

Dependent claims 2 to 8 concerned particular embodiments of the method according to claim 1. Independent claims 10 and 11 were directed to a kit containing first and second nucleotide probes.

II. With the statement of grounds of appeal dated 9 October 1998, the appellants (patentees) filed a main request (claims 1 to 17) and two auxiliary requests. A new

citation was provided.

Claim 1 of the **main request** was identical to claim 1 of the request before the opposition division (cf section I above). Claims 9 and 12 were as follows:

"9. A split probe hybrid comprising a detectable first nucleotide probe and a second nucleotide probe hybridised to adjacent segments of a target base sequence, the detectable first nucleotide probe being capable of linkage to the second nucleotide probe, characterised in that the detectable first nucleotide probe and/or the second nucleotide probe are hybridised to either side of a variant sequence associated with a disease state or to the corresponding normal sequence."

"12. A split probe hybrid comprising a detectable first nucleotide probe and a second nucleotide probe hybridised to adjacent segments of a target base sequence, the detectable first nucleotide probe being capable of linkage to the second nucleotide probe, characterised in that the detectable first nucleotide probe and/or the second nucleotide probe are hybridised to the target base sequence such that a variant base sequence associated with a disease state therein is at the terminal end of one of said probes, which terminal end is contiguous with the other of said probes or are hybridised to the corresponding normal sequence, wherein the length of the detectable first nucleotide probe and of the second nucleotide probe is such that it allows a hybridisation and selective denaturation of the linked probe above 60°C in aqueous solution."

III. The respondents (opponents) replied to the statement of grounds of appeal. They also provided a new citation.

- IV. On 2 July 2001, the board issued a communication with an outline of the points to be discussed at the oral proceedings and a provisional view on some of the issues.
- V. In reply to the board's communication, the appellants filed new first to third auxiliary requests. A new citation was also filed.
- VI. Oral proceedings took place on 11 October 2001. The appellants, while maintaining the main request already on file, submitted new first and second auxiliary requests in replacement of the auxiliary requests previously on file.

In the **first auxiliary request** (claims 1 to 11), claim 1 was as claim 1 before the opposition division (cf section I above) except that the feature "**by means of the linkage step**" was replaced by the feature "**by formation of a split probe hybrid which is subjected to linking to form a linked probe hybrid**". Claim 9 was identical to claim 9 of the main request (cf section II above).

In the **second auxiliary request** (claims 1 to 11), claim 1 read as follows:

"1. A method for discriminating between alternative nucleotide sequences, which method comprises subjecting adjacent segments of a target base sequence to hybridisation with a detectable first nucleotide probe and with a second nucleotide probe, to form a hybrid, the nucleotide sequence of the first and second probe being such that where they form a split probe hybrid

with a complementary target sequence they may subsequently be linked, subjecting any hybrid obtained to linkage, and detection of any hybrid obtained;

the DNA sequence of the detectable first nucleotide probe and of the second nucleotide probe being such that a potential mismatch in the target sequence lies between said probes;

the method being effected such that a complementary target sequence is discriminated from a target sequence with one or more non-complementary nucleotides."

Claim 9 was identical to claim 9 of the main request (cf section II above).

VII. The appellants indicated that several passages in the description of the application as filed supported the feature "by means of the linkage step" (cf claim 1 of the main request) and gave the skilled person the necessary information about its meaning: eg page 4, lines 1 to 11, lines 21 to 22; page 5, lines 7 to 16; page 6, line 25 to page 7 line 12; page 10, line 22 to page 15, line 20.

As for the feature "wherein the length of the detectable first nucleotide ... in aqueous solution" (cf claim 12 of the main request), it found support on page 9, lines 2 to 10 of the application as filed.

The feature "by formation of a split probe hybrid..." (cf claim 1 of the first auxiliary request) was supported by the passage bridging pages 9 and 10 and by page 12, lines 7 to 9 of the application as filed.

In their view, there was no offence against Article 123

EPC. Moreover, the method and the split probe hybrids claimed were clearly defined and sufficiently disclosed for a skilled person, in spite of some non-smooth definitions which were used. The examples indicated the suitable conditions for carrying out the assay as claimed.

They argued that the claimed subject-matter was distinguishable, and thus novel, over that described in document (1) because - as correctly seen by the opposition division - the latter disclosed a stringency-dependent assay, **not** a method which used the ligation step for discriminating between matched and mismatched probes. In fact, when reading the description of the patent specification, the skilled person derived that the claimed method was performed under non-stringency conditions: see the discussion on page 2, lines 45 to 50 and the results reported in Example 4 of experiments where non-stringency hybridisation temperatures were used relative to the melting temperature of each of the oligonucleotides. The features recited in the claim were indicative of a stringency-independent assay, namely formation of a hybrid under conditions regardless of whether the target sequence was matched or mismatched, placement of the potential mismatch between the two probes or at the terminal end thereof, and use of ligation as the point of discrimination. In contrast thereto, document (1) emphasized that stringency was the critical step for discrimination (see in particular pages 12 and 13 and page 20, lines 5 to 9) and relied on hybridisation rather than ligation for assaying the presence of a mismatch in the target sequence.

As regards the second auxiliary request, the appellants

maintained that, as the request was limited to the aspect of the invention (potential mismatch between the probes), which had not been disputed by the respondents upon opposition, it was not open to discussion. Thus, there were no reasons for a remittal to the first instance.

VIII. The respondents objected under Article 123(2) EPC to the feature "by means of the linkage step" in claim 1 and to the feature "wherein the length of the detectable first nucleotide ... in aqueous solution" in claim 12 of the main request. These two features were also objected to under Article 84 EPC as being unclear.

Furthermore, they argued under Article 83 EPC that the description did not disclose the steps which were required in order to achieve discrimination of a complementary target sequence from a target sequence with one or more non-complementary nucleotides "by means of the linkage step".

As regards novelty, they submitted in particular that the subject-matter of method claim 1 and of product claim 12 of the main request as well as that of method claim 1 of the first auxiliary request was not adequately distinguished over the subject-matter of document (1) as inter alia the conditions at which ligation had to take place did not preclude stringent conditions.

In respect of the second auxiliary request, they argued that it raised issues of clarity and lack of novelty which could be decided by the board. Thus, there were no reasons for remitting the case to the first instance.

IX. The appellants requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request (claims 1 to 17 filed with letter dated 9 October 1998) or on the basis of the first or second auxiliary request filed during the oral proceedings.

The respondents requested that the appeal be dismissed.

Reasons for the Decision

The main request: the feature "by means of the linkage step"

1. The feature "by means of the linkage step", which distinguishes claim 1 at issue from claim 1 as granted, has a limitative effect on the scope of protection and for this reason does not raise issues under Article 123(3) EPC.

2. However, the feature in question is objected to by the respondents under Articles 84 and 123(2) EPC as being meaningless and because, in their view, nowhere in the application as filed it is disclosed that discrimination between alternative target sequences can be effected through the ligation step. The latter objection is also presented as an objection of lack of sufficient disclosure under Article 83 EPC. Furthermore, in the respondents' view, the said feature does not distinguish the claimed method from that of document (1) (lack of novelty objection).

3. While it is true that nowhere in the application as filed the explicit statement is found that "the ligation step" as such is **the step** which allows

discrimination between the target nucleotide sequences, it is also a fact that ligation is one of the relevant steps of the method as disclosed in the patent specification. In the board's judgement, the key question here is whether the feature "by means of the linkage step" in the context of claim 1 contributes to a clear definition of the method for which protection is sought and to its distinction over the known method of document (1), which also involves a ligation step. As a matter of fact, the feature in question was introduced as an amendment into claim 1 as granted during the procedure before the opposition division in order to create a clear distinction over the disclosure of document (1). The opposition division indeed accepted that thereby a clear distinction was made, a finding which is still disputed by the respondents. Thus, the key issue here is essentially one of Article 84 EPC in consequence of the said amendment, the issue of novelty being, however, directly linked thereto.

4. Claim 1 at issue is directed to a method for discriminating between alternative nucleotide sequences which comprises essentially the steps of (i) subjecting adjacent segments of a target base sequence to hybridisation with a detectable first nucleotide probe and with a second nucleotide probe, to form a hybrid; (ii) subjecting any hybrid obtained to linkage, and (iii) detecting any hybrid obtained.

The claim specifies in general terms that the DNA sequence of the detectable first nucleotide probe and of the second nucleotide probe are such that a potential mismatch in the target sequence lies either between the said probes or at the terminal end of one

of said probes which is contiguous with the other of the said probes.

Finally, the claim indicates also in general terms that the method is to be effected such that a complementary target sequence is discriminated from a target sequence with one or more non-complementary nucleotides by means of the linkage step.

5. It is noted - for the sake of the following discussion - that the claim **does not** refer to any specific conditions for the hybridisation step (cf point 4, first paragraph, item i) above). **Nor does** the claim refer to any particular length of the probes. Moreover, it is also noted that the expression "a potential mismatch in the target sequence lies ... at the terminal end of one of said probes which is contiguous with the other of the said probes", which characterises one of the possible embodiments of the method, **does not** necessarily confine the mismatch to the last nucleotide of the probe, as the term "at the terminal end" identifies the region, **not** the exact location.

6. The description of the patent specification in the several passages referred to by the appellants (cf section VII above, first paragraph) outlines with sufficient details the rationale of the method for discriminating between alternative nucleotide sequences. In essence, the method is a multi-step process which relies on the less efficient ligation of the probes when a mismatch is present (cf page 3, lines 35 to 44) and on the change of thermal stability of the cross-linked probe over the corresponding probe hybrid alone (cf page 5, lines 23 to 25). In such a method there are different variables like the length of

the probes and the temperature of hybridisation and/or selective denaturation. The discriminatory specificity is achieved by careful control and tuning of these variables, the ligation being one of the essential steps involved in the method. However, ligation is not **the** step that per se directly allows discrimination.

7. In the board's judgement, claim 1 does not adequately reflect said rationale in terms of its essential technical features. As indicated above (cf point 4, third paragraph), the claim refers in general terms to the fact that "the method is to be effected such that a complementary target sequence is discriminated from a target sequence with one or more non-complementary nucleotides by means of the linkage step.". Such a general language does not properly subsume the rationale of the alleged invention in acceptable technical terms as the expression "effected such that" in combination with the feature "by means of the linkage step" does not define any specific manner of operating, the latter feature representing only a vague reference to a ligation step which has to be carried out at some stage and which is not directly linked to discrimination. The feature "by means of the linkage step" is per se meaningless. Thus, already for this reason, the claim is not allowable for lack of clarity. In addition, the said feature is inadequate to distinguish the method claimed over that disclosed in document (1).

8. The latter document is prior art within the meaning of Article 54(2) EPC for the embodiment of claim 1 in relation to the presence of the potential mismatch "at the terminal end of one of said probes", which is not entitled to the priority date as it is not covered by

the priority application, this being undisputed by the parties.

Document (1) discloses a nucleotide sequence detection method which comprises essentially the steps of **i)** subjecting adjacent segments of a target base sequence to hybridisation with a diagnostic nucleotide probe and with a contiguous nucleotide probe; **ii)** linking the two probes so as to form a probe complementary to the target sequence, and **iii)** detecting the resulting hybrid. In order to allow detection of single base pair mismatches in the target sequence, the diagnostic probe is preferably relatively short (4-5 base pairs), whereby inevitably the potential mismatch will lie at the terminal end (cf point 5, last sentence above). Such a method falls under the terms of the general wording of claim 1 at issue as it comprises the same steps (cf point 4, first paragraph above). The argument put forward by the appellants that document (1) discloses a stringency-dependent assay while claim 1 concerns a stringency-independent assay cannot be accepted because the wording of claim 1 does not refer to any specific conditions for hybridisation and in particular does not preclude stringency conditions (cf point 5, first sentence above).

9. For these reasons, the board considers that claim 1 fails to contain the essential technical features which distinguish it over the subject-matter disclosed in document (1). Thus, the claim, and consequently also the request of which it is part, cannot be allowed under the terms of both Articles 84 and 54 EPC.
10. Under these circumstances, it is not necessary to discuss the issues in relation to the other contested

feature "wherein the length of the detectable first nucleotide ... in aqueous solution" of claim 12 of the main request.

The first auxiliary request: the feature "by formation of a split probe hybrid which is subjected to linking to form a linked probe hybrid"

11. In this request, the feature "by means of the linkage step" has been replaced by the feature "by formation of a split probe hybrid which is subjected to linking to form a linked probe hybrid". In the board's judgement, this amendment does not overcome the problem of a clear definition of the claimed subject-matter and of its delimitation over the subject-matter of document (1). This is because the new feature substantially expresses in a different way what was already expressed in claim 1 of the main request by the feature "by means of the linkage step". It is merely the statement that linking of the split probe takes place, no elements being added which properly reflect in technical terms the rationale of the alleged invention (cf points 6 and 7 above). Thus, essentially for the same reasons put forward above in respect of the main request, the board considers that this claim, and consequently also the request of which it is part, cannot be allowed under the terms of both Articles 84 and 54 EPC.

The second auxiliary request

12. The claims of this request have been limited to the embodiment whereby the DNA sequence of the detectable first nucleotide probe and of the second nucleotide probe is such that a potential mismatch in the target sequence **lies between said probes** (cf section VI

above).

13. No objections under Article 123(2) and (3) EPC were raised by the respondents. Nor does the board have any such objections. Furthermore, the priority entitlement of this aspect of the invention is undisputed and thus document (1) constitutes prior art only under the terms of Article 54(3)(4) EPC.
14. The appellants maintain that the patentability of the aspect of the invention which is now claimed had never been questioned by the respondents upon opposition. Thus, in their view this aspect is not open to discussion.
15. The respondents argue that the amendments of the claims in view of their restriction to a particular aspect of the invention raises issues of lack of clarity and also lack of novelty, which, in their view, could be decided by the board.
16. Without entering into the merit of the above controversy, the board considers that, as in any case the inventive step issue has not yet been examined by the opposition division, it is appropriate to make use of the power granted to the board under Article 111(1) EPC to remit the case to the first instance for further prosecution on the basis of this request. Thereby all the controversial procedural and substantive issues in relation to the patentability of the claimed subject-matter can be decided by the first instance and the right to an appeal is maintained for use if appropriate. This is in line with the established principle of the case law that appeal proceedings should not be used as a continuation of the first

instance proceedings (cf eg T 47/90 OJ EPO 1991, 486, point 3), and furthermore ensures the parties' right to two instances.

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 for further prosecution on the basis of the second auxiliary request filed during oral proceedings.

The Registrar:

The Chairman:

P. Cremona

L. Galligani