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D E C I S I O N
of 19 March 2003

Case Number: T 0749/00 - 3.3.4

Application Number: 92114727.8

Publication Number: 0525821

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Methods and structures employing non-radioactive chemically-labelled polynucleotide probes

Applicant:
ENZO BIOCHEM, INC.

Opponent:
-

Headword:
Polynucleotide probes/ENZO BIOCHEM, INC.

Relevant legal provisions:
EPC Art. 56

Keyword:
"Main request, inventive step (yes)"

Decisions cited:
T 0455/91

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0749/00 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 19 March 2003

Appellant: ENZO BIOCHEM, INC.
60 Executive Boulevard
Farmingdale, New York 11735 (US)

Representative: VOSSIUS & PARTNER
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 28 January 2000 refusing European patent application No. 92 114 727.8 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: M. R. J. Wieser
V. Di Cerbo

Summary of Facts and Submissions

I. The patent application EP 92 114 727, publication number EP-A-0 525 821, with title "Methods and structures employing non-radioactive chemically-labelled polynucleotide probes", which is a divisional application of the EP application 84 100 836.0, was refused by the examining division under Article 97(1) EPC, as it was found that the claims of the main and auxiliary requests then on file did not meet the requirements of Article 54 EPC, or 56 EPC respectively.

II. The appellants (applicants) lodged an appeal against this decision. The final requests were that the decision under appeal be set aside and a patent be granted on the basis of the main request, claims 1 to 25, filed at the oral proceedings, or on the basis of the auxiliary request, claim 1, filed with letter dated 18 February 2003.

III. Claim 1 of the main request, filed at oral proceedings held on 19 March 2003 read as follows:

"A method for detecting a polynucleotide sequence which comprises:

(a) fixing or immobilizing said polynucleotide sequence to a glass or plastic substrate such that the polynucleotide sequence is in a single-stranded form and is capable of hybridizing to complementary nucleic acid sequences;

(b) forming a duplex comprising said single-stranded polynucleotide sequence hybridized to an oligo- or polynucleotide probe, said probe having attached thereto a non-radioactive chemical label comprising a signalling moiety capable of generating a quantifiable

signal detectable by means selected from photometric techniques, spectrophotometric techniques, visually, colorimetric techniques, chemiluminiscent techniques, fluorometric techniques and immunofluorometric techniques;

(c) generating said quantifiable signal; and

(d) detecting said quantifiable signal thereby detecting said polynucleotide sequence."

Dependent claims 2 to 14 referred to preferred embodiments of the method of claim 1. Independent claims 15 and 16, and claims 17 to 22 dependent thereon, referred to "a method for determining the quantifiable presence of a selected genetic material". Independent claim 23 differed from claim 1 in item (b), where a signal was generated "that can be detected in solution". The method of independent claim 24 and dependent claim 25 differed from the method of claim 1 in that the order of items (a) and (b) was changed.

All independent claims contained the technical features that a polynucleotide, or genetic material, either in single stranded form or as duplex (claim 24), is fixed or immobilized to a glass or plastic support, and that a probe having attached thereto a non-radioactive chemical label is used for its detection.

IV. The following documents are referred to in this decision:

(1) EP-A-0 063 879

(2) Methods in Enzymology, vol. 68, 1979, pages 419 to 429

(3) GB-A-2 014 727

V. The arguments of the appellants may be summarized as follows:

The method claimed according to the main request was not made obvious by document (1). This document, while disclosing chemically-labelled polynucleotide probes as alternative to prior art radioactively labelled probes, for use in *in-situ* hybridisation and conventional blotting methods, does not contain information that would prompt a skilled person to modify said conventional methods by using plastic or glass surfaces as supports for immobilizing the polynucleotides to be detected. The use of these materials, although known as support for the immobilization of proteins, was not known or suggested in any prior art document at the relevant date of the application, i.e. January 1983, for the fixing or immobilization of nucleic acids. The use of glass and plastic substrates allowed the provision of a rapid assay for a large number of samples which is easy to automate.

Reasons for the Decision

1. The subject-matter of the claims of the main request is fully supported by the earlier application as originally filed, in particular claims 1 to 37 and pages 20 to 22 ("summary of the invention"), where all technical features of the claimed method are disclosed. For the additional feature of claim 23, "a signal that can be detected in solution", a basis can be found on page 53, lines 1 to 3 or page 55, line 29 to 33, and for the feature of claim 24, i.e. duplex formation before immobilization, on page 55, lines 1 to 12.

The main request is thus in accordance with the requirements of Article 76(1) EPC.

2. As none of the documents presently on file discloses the same subject-matter as claimed, novelty according to Article 54 EPC is acknowledged.

3. As regards the issue of inventive step (Article 56 EPC), the Board considers document (1) to be the closest state of the art.

3.1 This document discloses chemically labelled nucleotides to be used as alternatives for probes carrying radioactive labels in methods for the detection of nucleic acid components (page 4, lines 13 to 18; page 8, lines 28 to 32). Starting on page 9, line 11, several criteria are listed that have to be satisfied by such modified nucleotides in order to be suitable as a substitute for radioactively-labelled probes. These criteria are, *inter alia*, high specificity and sensitivity, stable hybridisation to moieties to be detected, and physical and biochemical properties that do not require that current procedures using radioactive hybridization probes need to be extensively modified.

On page 33, a general protocol for probe detection *via in situ* colony, or northern/southern hybridisation methods, using the disclosed chemically-labelled nucleotides is illustrated. In Example 9 (starting at page 51), several embodiments of *in situ* hybridisation processes are described. On page 34 reference is made to non-in situ methods (lines 9 to 14 and 29 to 31).

3.2 In the light of the disclosure in the closest state of the art, the objective technical problem to be solved can be seen in the provision of an alternative method for the detection of a polynucleotide sequence by use

of non-radioactively labelled probes, which, without loss of specificity and sensitivity, allows a simple and rapid quantitative detection of a high number of samples.

3.3 This problem is solved by the subject-matter of independent claims 1, 15, 16, 23 and 24 of the main request, which is distinguished from the disclosure in document (1) such, that the polynucleotide, or genetic material, to be detected is fixed or immobilized to a glass or plastic support.

This is in contrast to northern or southern blotting techniques, referred to in document (1), in which a print of electrophoretically separated DNA or RNA fragments is transferred by "blotting" to a specific filter paper or membrane, where they are detected by hybridisation with homologous, labelled probes. This technique, using radioactively-labelled DNA probes, is described in document (2), page 421.

On page 50, lines 7 to 15 of the present application, it is said, that it is highly desirable in the practice of the invention, that the genetic material to be identified, be rapidly fixed to a substrate, as this would allow rapid testing of numerous samples.

3.4 A major point to be considered is whether the skilled person at the priority date of the present application, i.e. January 1983, in order to solve the problem underlying the application, was aware of information, which would have prompted him to modify the disclosure in document (1) and to arrive at the claimed subject-matter, by fixing or immobilizing the nucleic acids to be detected to a glass or plastic substrate, in an obvious way.

3.5 It is not disputed that the immobilization of proteins to glass substrates was known to the skilled person at the relevant date. This is acknowledged on page 50, lines 1 to 6 of the description, where prior art references are indicated referring to the detection of enzymes, antibodies and antigens immobilized to various substrates such as siliceous materials.

Document (3) discloses the immobilization of "an immunologically active substance" to a frosted glass. In the passage bridging pages 1 and 2 a list of substances to be conjugated to the glass surface is given. The list, containing a broad spectrum of entities such as proteins, antigens, hormones, immunglobulins, bacteria, viruses, protozoa, and antibodies, **does not mention** polynucleotides.

3.6 When deciding on the obviousness of transferring this immobilization technique to the immobilization of nucleic acids, the attitude of the notional skilled person in the field of biotechnology has to be considered, which is defined by the case law of the Boards of Appeal, e.g. in decision T 455/91 (OJ 1995, 684) such, that the skilled person's attitude is considered to be conservative. He would not go against an established prejudice, nor try to enter unpredictable areas nor take incalculable risks. He would perform a transfer of technology from a neighbouring field to his special field of interest, if this transfer involved routine experimental work comprising only routine trials.

3.7 The closest state of the art, document (1), disclosing chemically-labelled nucleotide probes and detection assays using them, states on page 10, lines 10 to 16,

that it is an essential criterium of the probes, that their physical and chemical properties are such "that current procedures using radioactive hybridization probes need not to be extensively modified".

This statement is considered as instructing the reader that no major deviation from prior art assay protocols is necessary when the probes according to document (1) are used. It would not be interpreted by a conservative and cautious skilled person, as defined in the case law of the Boards of Appeal, to be an invitation to modify the methods known from the prior art, like document (2), for the detection of polynucleotide sequences, and to apply a different technology. All the more as document (3), disclosing this technology, while containing a comprehensive list concerning its applicability, does not mention the immobilization of nucleic acids at all.

3.8 The Board comes to the conclusion, that a cautious and conservative skilled person, who is aware of the prior art documents on file, in order to solve the problem underlying the invention, would not be prompted to modify the teaching of the closest prior art, document (1), and to arrive at the subject-matter of claims 1 to 25 of the main request in an obvious way. Therefore, the claims are based on an inventive concept and meet the requirements of Article 56 EPC.

4. The description of the application contains on pages 23 to 49 a list of items (1) to (109), which are designated as being "preferred embodiments of the present invention". The function of these items, which contain various subject-matter not contained in the claims of the main request, is not clear. Said items seem to be in contradiction to the requirements of Rule 29 EPC, a matter which has to be considered when 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 first instance with the order to grant a patent on the basis of claims 1 to 25 filed at the oral proceedings as main request, and a description to be adapted thereto.

The Registrar: The Chairwoman:

P. Cremona U. M. Kinkeldey