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D E C I S I O N
of 1 December 2000

Case Number: T 0332/98 - 3.3.4

Application Number: 90124738.7

Publication Number: 0435150

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Amplification capture assay

Applicant:
ENZO BIOCHEM, INC.

Opponent:
-

Headword:
Capture assay/ENZO

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step (no)"

Decisions cited:
T 0197/96, T 0784/96, T 0870/97

Catchword:
-



Case Number: T 0332/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 1 December 2000

Appellant: ENZO BIOCHEM, INC.
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Decision under appeal: Decision of the Examining Division of the
European Patent Office posted 7 November 1997
refusing European patent application
No. 90 124 738.7 pursuant to Article 97(1) EPC.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: L. Galligani
S. C. Perryman

Summary of Facts and Submissions

I. The applicants lodged an appeal against the decision of the examining division issued on 7 November 1997 whereby the European patent application No. 90 124 738.7 (published as EP-A- 435 150) was rejected. Basis of the rejection were claims 1 to 9. The examining division decided that claim 1 offended against Article 123(2) EPC. Moreover, it was found that claim 1 was unclear and that the claimed subject-matter lacked an inventive step having regard to

(1) EP-A- 0238 332

in combination with either

(4) Proc. Natl. Acad. Sci. USA, Vol. 86, February 1989, pages 1173 to 1177; or

(5) Medical Biology, Vol. 64, 1986, pages 1 to 12.

II. With the statement of grounds of appeal, the appellants filed a new request.

III. On 25 July 2000, the board issued an official communication pursuant to Article 11(2) of the rules of procedure of the boards of appeal with a preliminary opinion on the points to be discussed.

IV. On 31 October 2000, the appellants filed new claims 1 to 7 in reply to the board's communication.

Independent claim 4 thereof read:

"A method for the determination of single-stranded

target nucleic acid in a test sample, which method comprises:

- (a) contacting the test sample, under conditions permissive of amplification, with a nucleic acid polymerase capable of amplifying the copy number of the target nucleic acid;
- (b) contacting under conditions permissive of amplification the test sample and any amplified copy number of the target nucleic acid so formed with a first polynucleotide probe comprising a first universal label-capturing moiety attached to a first single-stranded polynucleotide segment, the first single-stranded polynucleotide segment being hybridizable with a first portion of the target nucleic acid so as to form a hybrid wherein the universal label-capturing moiety is one partner of a specific binding pair and, which further comprises, thereafter contacting the reaction product thereof with a detectably labeled binding partner for the universal label-capturing moiety;
- (c) contacting, under conditions permissive of hybridization, any hybrid so formed with a matrix-affixed second polynucleotide probe comprising a second single-stranded polynucleotide segment hybridizable with a second portion of the target nucleic acid so as to form a bound complex;
- (d) separating, if necessary, the bound complex from any unbound nucleic acid; and
- (e) determining the presence or absence of the

amplified target nucleic acid by capturing and observing the presence or absence of the universal label."

Dependent claims 5 to 7 concerned particular embodiments of the said method. Product claims 1 to 3 were directed to means for carrying out such a method.

- V. Oral proceedings took place on 1 December 2000. The appellants submitted essentially that none of the documents (1), (4) and (5) provided any motivation to develop the invention as claimed. Although document (1) differed from the assay method as claimed only in that the second probe was not fixed on the support so that hybridisation could take place in a liquid-liquid sandwich system, it dismissed liquid-solid sandwich hybridisation as an unsuitable technique and thus taught away from the claimed invention. Also document (5) motivated the skilled person away from mixed-phase sandwich hybridisation as it described it as having an inadequate detection sensitivity (cf page 9) and it pointed to problems caused by re-annealing of target DNA (page 5). Document (4) did not disclose indirect labelling and by relying for amplification on a transcription-based amplification system (TAS) as an alternative to PCR (cf page 1176) taught away from the method as claimed. Further the method of document (4) using a TAS system took far longer, of the order of 3 to 4 hours with each amplification cycle taking only 20 to 25 minutes, than that of the present invention where using PCR each amplification cycle might take only a couple of minutes, and labelling only a few additional minutes. Accordingly the invention showed an improvement.

VI. The appellants requested that the decision under appeal be set aside and that a patent be granted on the basis of the claims filed 31 October 2000.

Reasons for the Decision

1. The set of claims at issue gives rise to certain objections to clarity of the terms put forward, but these do not affect the critical issue of what distinguishes claim 4 from the prior art, and whether inventive step can be acknowledged for this claim. Accordingly this critical issue is dealt with immediately.

2. The essence of the method for determining a single-stranded target nucleic acid according to independent claim 4 at issue lies in combining amplification of target nucleic acid sequences with solid-liquid sandwich hybridisation. To this extent, the claim proposes carrying out the following steps:
 - (a) amplifying the nucleic acid with a polymerase;

 - (b) contacting the amplified nucleic acid, under conditions permissive of hybridisation, with a first probe comprising a label-capturing moiety;

 - (c) contacting, under conditions permissive of hybridisation, the so formed hybrid with a second probe which is fixed on a matrix;

 - (d) separating bound from unbound;

 - (e) determining the presence or absence of target

nucleic acid on the matrix by capturing the label.

3. The closest prior art is represented by document (1) which describes a method for detecting a nucleic acid which comprises the steps of:
 - contacting, under hybridisation conditions, single-stranded nucleic acid with at least two probes, one of them being a reporter probe linked to a label moiety, the other one being a support-binding probe which fixes on a support after hybridisation has taken place;
 - separating bound from unbound;
 - detecting the presence or absence of the hybrid formed and fixed on the support by means of the label moiety.

The document states on page 7, lines 37 to 40 that the nucleic acid to be determined can be amplified before it is hybridised in order to increase sensitivity.

4. According to the case law of the boards of appeal (cf eg T 197/96 of 26 April 1999, T 784/96 of 15 July 1999 and T 870/97 of 6 July 2000), for the board to be able to recognize that the claimed subject matter achieves an improvement over the prior art, there must be evidence that what is claimed achieves an improvement over the closest prior art, here that described in document (1). There is no such evidence. The appellant has submitted that the use of PCR amplification, as described in the application in suit, will allow a faster assay than the TAS amplification system of document (4). But document (4) is not considered as the

closest prior art, and the comparison is in any case not legitimate because claim 4 does not exclude the use of TAS amplification. The difference in speed thus cannot be considered an improvement achieved by the claimed invention. In the light of document (1), the underlying technical problem can thus only be defined as the provision of an alternative form of assay for determining a single-stranded target nucleic acid.

5. The solution proposed is the method of claim 4 as outlined in point 2 above. Admittedly, the only difference between the claimed method and that of document (1) lies in that, while according to claim 4 the second probe is fixed to the matrix (liquid-solid sandwich hybridisation), the prior art teaches fixing the formed hybrid on the support via the second probe after hybridisation (liquid-liquid sandwich hybridisation).
6. The relevant question is whether the skilled person, in consideration of other prior art, in seeking an alternative, would have arrived at a modification of the arrangement specifically taught in document (1) by using, instead of a support-binding probe, a support-bound probe.
7. In this respect, the appellants' view is that the skilled person had no motivation to modify the liquid-liquid hybridisation method described in document (1) so as to transform it into a liquid-solid method because the document itself dismissed liquid-solid hybridisation as an unsuitable technique being slow, inefficient and difficult to perform in automation. In their view, also document (5) confirmed this by pointing on page 5 to the problem of re-annealing of

the nucleic acid, and on page 9 to the slow reaction rate and inadequate detection sensitivity. They conclude that the prior art actually taught away from liquid-solid sandwich hybridisation.

8. For the board to be able to recognize that there existed a prejudice against using liquid-solid hybridization, there would have to be evidence that this was the general opinion of skilled persons in the art. In accordance with the established case law (cf Case Law of the Boards of Appeal of the European Patent Office, 3rd edition, I-D, item 7.2) such a prejudice cannot be established by reference to a single patent document, but should be established as being generally taught in text-books or the like at the time in question. The reference by the appellant to document (1) is quite inadequate to establish a prejudice in this sense. In fact, many different types of liquid-solid sandwich hybridisation were known in the art (cf eg page 2, lines 10 to 24 of document (1); cf also the introductory part of the patent in suit). The drawbacks referred to in document (1), namely hybridisation kinetics and difficulty in automation, in respect to some types of liquid-solid hybridisation (ibid., page 2, lines 14 to 24) do not reflect a widespread dislike in the art for this hybridisation approach. The pointer to drawbacks is used as a premise for illustrating the benefits of the liquid hybridisation sandwich assay described in the document, which is said to "avoid the disadvantages of solely liquid-solid hybridisations and solely liquid hybridisations and combine the advantages of both" (ibid., page 3, lines 28 to 29). The latter citation shows also that some advantages were seen in a liquid-solid sandwich hybridisation system. Thus in

document (4), of a date some two years later than document (1), bead-based sandwich hybridization, a particular form of liquid-solid sandwich hybridization, and the comment is made (cf page 1176) "The ability to carry out quasi-homogeneous hybridizations (i.e. near solution-like hybridization conditions) by using bead-bound oligonucleotides as a hybridization matrix has permitted the rapid detection of the TAS-amplified HIV-1 RNA product." Far from showing any generally held prejudice against liquid-solid sandwich hybridization, document (4) shows that some forms of this were considered as equivalents of liquid-liquid sandwich hybridization. Other aspects of improving the low sensitivity of sandwich hybridization methods in general are addressed also in document (5) which indicates that procedures to amplify the nucleic acid sequences to be detected is a potential solution to the problem of low sensitivity. In this document, both the re-annealing problem as well as the slow reaction rate are put in relation with the low amount of the target material.

9. As for document (4), where - as already indicated above - liquid-solid hybridisation is used, the argument by the appellants that it would have taught away from the claimed method because amplification is carried out by a time-consuming TAS method is not persuasive because, admittedly, the use of TAS in step (a) is not excluded by claim 4.

10. In the board's judgement, the skilled person, having learnt from document (1) that the sensitivity of a liquid-liquid sandwich hybridisation assay could be increased by amplifying the target nucleic acid sequence in the test sample before it is hybridised,

when looking for an alternative form of the assay, would have readily thought of a liquid-solid sandwich hybridisation assay preceded by an amplification step. The use of a support-bound probe, instead of a support-binding probe, would have been for the skilled person an obvious measure to adopt. Thereby the skilled person would have arrived at an assay system falling within the scope of claim 4, which thus lacks an inventive step. Consequently, the sole request on file of which claim 4 is part is not allowable under Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairperson:

U. Bultmann

U. Kinkeldey