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D E C I S I O N
of 11 February 1999

Case Number: T 0289/96 - 3.3.4

Application Number: 89302331.7

Publication Number: 0332435

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Method of detecting nucleotide sequences

Patentee:
Zeneca Limited

Opponent:
F. Hoffmann-La Roche & Co. Aktiengesellschaft

Headword:
Detecting sequences/ZENECA

Relevant legal provisions:
EPC Art. 56, 83

Keyword:
"Sufficiency of disclosure (yes)"
"Inventive step (yes)"

Decisions cited:
T 0409/91, T 0435/91, T 0694/92

Catchword:
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Case Number: T 0289/96 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 11 February 1999

Appellant: (Opponent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 18 January 1996 rejecting the opposition filed against European patent No. 0 332 435 pursuant to Article 102(2) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: F. L. Davison-Brunel
C. Holtz

Summary of Facts and Submissions

I. The appeal lies from the decision of the opposition division issued on 18 January 1996 by which the opposition against the European patent No. 0 332 435 (European patent application No. 89 302 331.7) filed under Article 100(a) and (b) EPC, was rejected.

II. Independent claim 1 of the patent in suit read as follows:

"A method for detecting the presence or absence of at least one variant nucleotide in one or more nucleic acids contained in a sample, which method comprises:-

treating the sample, together or sequentially with appropriate nucleoside triphosphates, an agent for polymerisation of the nucleoside triphosphates and a diagnostic primer for a diagnostic portion of a target base sequence under hybridising conditions, the nucleotide sequence of the said diagnostic primer being such that it is substantially complementary to the said diagnostic portion, the 5' or 3' terminal nucleotide of the diagnostic primer being either complementary to the suspected variant nucleotide or to the corresponding normal nucleotide, whereby an extension product of the diagnostic primer is synthesised when the said terminal nucleotide of the diagnostic primer is complementary to the corresponding nucleotide in the target base sequence, no extension product being synthesised when the said terminal nucleotide of the diagnostic primer is not complementary to the corresponding nucleotide in the target base sequence; and detecting the presence or absence of the suspected variant nucleotide from the

presence or absence of an extension product."

Dependent claims 2 to 9 concerned embodiments of the method according to claim 1. Claims 10 to 12 were directed to a nucleotide sequence for use in a method according to claims 1 to 9; claim 13 to a set of two nucleotide sequences according to claims 10 to 12; claims 14 and 15 to a kit for use in a method according to claims 1 to 9 and claim 16 to the use of a nucleotide sequence or set according to claims 10 to 13.

III. Of the documents cited during the opposition phase the following are referred to in the present decision:

(2) "DNA Replication", A. Kornberg, 1980, W.H. Freeman and Co., San Francisco, USA, page 96;

(3) Biochemistry, Vol. 20, 1981, pages 4570 to 4578;

(4) J. Clin. Invest., Vol. 71, 1983, pages 775 to 779;

(5) EP-A-0 123 513;

(6) Science, Vol. 230, 1985, pages 1350 to 1354;

(7) Biochimie, Vol. 67, 1985, pages 755 to 762;

(8) Nature, Vol. 324, 13 November 1986, pages 163 to 166;

(9) EP-A-0 237 362;

(10) The New England J. Med., Vol. 316, No. 11,

12 March 1987, pages 656 to 661;

(13) Nucl. Acids Res., Vol. 11, No. 20, 1983, pages 7251 to 7260;

(15) "From Genes to Clones: Introduction to Gene Technology", E-L. Winnacker, 1987, VCH, Weinheim (DE), pages 41 to 42.

IV. The opposition division considered that the claimed method was enabled by the description, that it was novel over documents (2) and (3) and involved an inventive step having regard to the teaching of document (9) (closest prior art) in combination either with that of documents (2) and/or (3) or with that of documents (13) and/or (15) or, furthermore, with that of documents (4) and/or (7). Documents (5) (6), (8) and (10) were also considered not to affect the inventiveness of the method claimed. The allowability of claims 10 to 16 was considered to depend directly on the patentability of the method claims 1 to 9.

V. With the statement of grounds of appeal, the appellants (opponents) filed the following new document:

(21) Science, Vol. 239, 29 January 1988, pages 487 to 491.

VI. The respondents (patentees) replied to the submissions by the appellants.

VII. On 27 October 1998, the board issued a communication with preliminary observations on the case.

VIII. In reply thereto, the respondents filed an auxiliary claim request. The appellants also filed further submissions.

IX. During oral proceedings, which took place on 11 February 1999, all previous claim requests were withdrawn and a main request consisting of method claims 1 to 9 as granted was submitted together with the amended description pages 2, 6, 7, 10 and 11.

X. The appellants argued that claim 1 failed to recite features which were essential to make the diagnostic method work, these being a control reaction for false results, at least a further primer and an amplification reaction, for example by PCR. According to the European case law, in particular to decision T 409/91 (OJ EPO 1994, 653), mandatory features had to be in a claim. Example 5, referred to by the respondents as a support for claim 1, had nothing to do with a real-life diagnostic situation as it was a model example in which cloned DNA was taken, **not** a biological sample. In any case, the results of this example in Figure 12 were not readable as the figure showed only smears. It was also observed that Figures 7 and 8 in relation to Examples 2, 3 and 4 were not readable and thus the skilled person could not derive therefrom any useful information. Moreover, the patent specification conveyed the mistaken teaching that polymerases with proofreading activity could also be used (cf page 7, line 52). Thus, the information available from the specification was not sufficient to enable the skilled person to achieve the desired result within the whole ambit of the claim (cf inter alia decision 435/91, OJ EPO 1995, 188).

As for inventive step, the appellants maintained that the properties of polymerases, in particular their capability to distinguish between paired and unpaired primers, were known in the art (cf eg documents (2) and (3)). Against this background, a number of documents had provided examples of how a genetic disease could be spotted by elongating selectively primers which matched perfectly (cf eg documents (5) and (6)). In particular, document (6), which was seen as the closest prior art, allowed a distinction between ß- and ä-globin genes based on the use of a primer (PC04) which was not elongated when the target sequence was the ä-globin gene, which did not pair with the 3'terminus of the primer. In view of the teaching of document (6), the skilled person would have readily recognised that the approach described therein in relation to the distinction between ß- and ä-globin genes was also applicable to situations in which a normal gene or the same gene containing a variation was present in a sample. Document (21) demonstrated that the skilled person was aware that selective primer elongation could be achieved by using eg Taq-polymerase.

XI. The respondents argued that the appellants had raised no serious doubts as to the possibility of performing and reproducing the invention as claimed in claim 1. The method had not necessarily to be carried out on a biological sample (eg blood), but could be carried out eg on DNA extracted from blood. Amplification was not an essential feature, but only an additional step. As regards the polymerases referred to in the specification, modified versions of proofreading polymerases were also available which had no proofreading activity. The skilled person knew from the

description in the patent specification that only such polymerases were usable. No further details were necessary.

As for inventive step, they submitted that, in their view, document (9) represented the closest prior art. This document, however, did not involve a selective elongation of primers with a mismatch at the 5' or 3' end and required further detection steps. The authors of documents (6) and (21) did not attach any diagnostic significance to individual mismatches in their primers and did not refer to any selective chain elongation. None of these documents, alone or in combination with other prior art documents (eg documents (2) or (3)), rendered obvious the method as claimed.

XII. The appellants requested that the decision under appeal be set aside and that the patent be revoked.

The respondents requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request submitted in the oral proceedings and the amended pages to the description and the remaining pages of the description as granted, and the drawings as granted.

Reasons for the Decision

Formal admissibility under Article 123(2) and (3) EPC

1. The sole claim request on file consists of method claims 1 to 9 as granted against which no objections under Article 100(c) EPC have been raised. The amended

description pages 2, 6, 7, 10 and 11 have been adapted to this limited set of claims. By these amendments no new matter has been generated. The appellants had no objections thereto. The formal requirements of Article 123(2) and (3) EPC are met.

Sufficiency of disclosure (Article 83 EPC)

2. Article 83 EPC requires that the invention be disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. Article 84 EPC specifies inter alia that the claims, which define the matter for which protection is sought, should be supported by the description. In the European case law there are several examples of cases in which questions of sufficiency and support were decided (cf eg T 409/91 (supra), T 694/92, OJ EPO 1997, 408). These, however, are questions which have to be examined in each case on its own merits. There is general agreement that, as pointed out eg in decision T 409/91 (supra, cf point 3.5 of the reasons), the purpose of the requirement of support by the description, insofar as its substantive aspect is concerned, and of the requirement of sufficient disclosure is the same, namely to ensure that the extent of protection conferred by the granted claims is justified by the actual technical contribution to the art. This implies inter alia, firstly, that a claim may not encompass subject-matter which is not sufficiently disclosed within the meaning of Article 83 EPC as it cannot be performed without undue burden and, secondly, that a claim should contain all the essential features of the invention which are necessary to meet the requirement of sufficient disclosure. These are exactly the issues

raised by the appellants in the present case.

3. The description of the patent in suit reveals that the invention stems from the realisation that "...by selecting the nucleotide sequence of an oligonucleotide primer appropriately it is possible to selectively achieve primer extension of either a sequence containing a suspected variant nucleotide or the corresponding sequence containing the normal nucleotide or to prevent such primer extension thus substantially simplifying the detection procedures necessary." (cf page 3, lines 16 to 19).

4. Accordingly, the skilled person is taught by the description a method whereby, when the presence or absence of one or more variant nucleotide sequence has to be detected in a sample, this is treated, together or sequentially, with appropriate nucleoside triphosphates, an agent for polymerisation of the nucleoside triphosphates and a diagnostic primer for a diagnostic portion of a target base sequence under hybridising conditions, and then the presence or absence of the suspected variant nucleotide is detected based on the presence or absence of an extension product. Guidance is provided on how to design the nucleotide sequence of a suitable primer: its length may be from about 5 to 50 bp; it should be substantially complementary to the diagnostic portion of the target sequence, the 5' or 3' terminal nucleotide of the primer being either complementary to the suspected variant nucleotide or to the corresponding normal nucleotide, so that an extension product of the primer is synthesised **only when** the terminal nucleotide of the primer is complementary to

the corresponding nucleotide in the target base sequence, and **not when** the terminal nucleotide of the primer is not complementary to the corresponding nucleotide in the target base sequence (cf page 3, lines 20 to 32). The reader is also told that "any extension product obtained may **if desired** be amplified by the polymerase chain reaction (PCR) as described in ..." (emphasis added) (cf page 3, lines 41 to 48). Details about the agent for polymerisation which may be used are given on page 7, lines 50 to 56. The description provides the definition of the different terms, information about the optimal length of the primer, an outline of the preferred embodiments, a table pointing to the relevant mutations in a series of known genetic disorders to be taken into account in designing the respective diagnostic primers, and examples in which the applicability of the approach as described is shown with reference to the figures (NB: as noted by the appellants, some of the figures in the printed patent specification are not as clear as those originally filed with the application. However, this is a practical problem linked to the reproduction of the document which has nothing to do with sufficiency of disclosure. Access to the original figures is possible through inspection of the file).

In all the examples, save Example 5, an amplification step is used. Example 5 is a model example in which two cloned DNA sequences are used, one containing a "normal" sequence and the other containing the "variant" sequence. This example reports the detection of the product when a matched primer is used in accordance with the approach described in the specification without amplification.

5. The wording of claim 1 (see Section II supra) outlines the same operational steps as the basic teaching of the patent in suit. It characterises the invention in the broadest outline as disclosed in the patent specification.

6. The claim does not need to include technical details which are manifest in the light of the overall disclosure or common general knowledge, such as eg the need for a control reaction, indications in respect of the length of the primer, nor features which characterise particular embodiments.

The amplification step is presented in the patent specification as an additional step (cf page 3, lines 41 to 45 of the patent specification) and no need is seen to include it in the main claim. In respect of the appellants' objection that without this operational step the method of claim 1 does not work in a real-life diagnostic situation, it is observed that:

- Claim 1 is generally directed to a method for detecting the presence or absence of at least one variant nucleotide in one or more nucleic acids contained **in a sample**, which does not necessarily

always mean a clinical sample where the target sequence can be present in very small amounts together with many other interfering substances so as to render amplification desirable, if not necessary. As a matter of fact, the method of claim 1 is meant to be broadly applicable also to simpler samples (cf Example 5) or to technical situations where the target sequence has undergone previous isolation or enrichment;

- The skilled person is able, on the basis of the description and of common technical knowledge to recognise technical situations in which amplification is necessary (possibly the great majority of the real-life diagnostic situations) and those in which amplification is not necessary. No undue burden is placed on the skilled person by leaving this option open, as done by the wording of claim 1.

The same rationale applies also to the use of a second primer which constitutes a further embodiment of the general approach outlined in claim 1 (cf claim 3).

7. As regards the alleged misleading information about the polymerases which can be used, the board considers that, as it is a fundamental teaching of the patent specification that elongation should take place only when the 5' or 3' terminal nucleotide of the primer matches the relevant nucleotide of the target sequence (cf points 4 and 5 supra), the skilled person is thereby unambiguously instructed to avoid the use of polymerases with proofreading activity.

8. For these reasons, the board considers that the information provided in the description of the patent specification is sufficiently clear and complete to enable a skilled person to carry out the method as claimed without undue burden. The requirements of Article 83 EPC are therefore satisfied.

Inventive step (Article 56 EPC)

9. In the board's judgement, the **closest prior art** is represented by document (9) which is also cited in the body of the specification (cf page 2, line 27 to page 3, line 12). This document, like the patent in suit, is concerned with a method for detecting the presence or absence of at least one nucleotide variation in one or more nucleic acids contained in a sample. The sample is repeatedly treated with primers (one for each strand of each nucleic acid), nucleotide triphosphates, and an agent for polymerization of the triphosphates, and then denatured, in a process wherein the sequence containing the nucleotide variation, if present, is amplified. The primer or primers are selected so as to be substantially complementary to each nucleic acid strand containing each different variation, such that the extension product synthesized from one primer, when it is separated from its complement, can serve as a template for synthesis of the extension product of the other primer. The detection step involves either the use of a labelled sequence-specific oligonucleotide probe and/or the use of a specific restriction endonuclease and/or use of direct sequencing methods on the amplified DNA.

10. In the board's opinion, document (6), selected by the

appellants as the closest prior art, is no more relevant than document (9), which also refers to it (cf sentence bridging pages 1 and 2 of document (9)), for the reasons given hereinafter:

The document in question relates essentially to the diagnosis of sickle cell anemia by an oligomer restriction method the sensitivity of which is enhanced by way of amplification of ß-globin DNA sequences by use of primers and DNA polymerase. The appellants have drawn the board's attention to the description of the primer PC04 (cf ibidem, Figure 1) which, in their view, is used to distinguish between the ß- and ä-globin genes based on a mismatch at the 3' terminus. However, apart from the fact that in Figure 1 more than one nucleotide difference between the ß- and ä-globin genes is indicated, no particular emphasis being placed on the mismatch in question, nothing in the text of the document points specifically to the mismatch at the 3' end of PC04 as being in any way significant for selective detection of the ß- or ä-gene based on primer elongation. Primer PC04, which is also described in document (9), is only one of the two primers used to amplify the ß-globin gene segment containing the relevant restriction site in view of the oligomer restriction analysis.

11. In view of document (9), the underlying technical problem is defined as being the finding of an alternative, possibly simpler, method for detecting at least one single base difference in nucleic acids.

12. As a solution thereto, the claims at issue propose a method based on the determination of the elongation or

non-elongation of a primer so designed that its 5' or 3' terminal nucleotide is either complementary to the suspected variant nucleotide or to the corresponding normal nucleotide in the target sequence. The examples show that, as mismatches at the 3' end prevent the elongation by polymerases, the method can indeed be used for detection of variant nucleotides in nucleic acids.

13. It has been argued by the appellants that, as the skilled person was aware of the fact that base pair matching was necessary for polymerisation to occur (cf documents (2) or (3)), the skilled person would have readily arrived at the claimed method, especially in the light of the prior art knowledge on how a genetic disease could be spotted by using an oligonucleotide with a mismatch (cf documents (5) and (6)) as well as in the light of the knowledge that selective primer elongation could be achieved with eg Taq polymerase (cf document (21)).

14. Various documents in the art dealt with methods for detecting specific nucleotide variations in nucleic acids, in particular in relation to genetic diseases. Documents (6) and (9) were among them. Their contents have already been discussed above (cf points 9 and 10). Nothing in these two documents would have provided a hint in the direction of the method claimed as both of them were essentially dealing with the amplification of the nucleotide sequence **containing** the variation to be detected in view of the subsequent detection step, be that hydridisation with a labelled probe or restriction analysis etc.

Document (5) was also known to the skilled person. This document proposed a method of detection based on forming a hybrid between a labelled probe and the target sequence in a position adjacent to the mutated nucleotide, adding a nucleotide derivative which, if complementary to the mutated base, protects the probe from digestion, and observing the presence or absence of the label attached to the target. This document explicitly draws the reader's attention to the need to use faithful polymerases free of exonuclease activity, like the calf thymus DNA polymerase (cf page 10, lines 24 to 36). The essential objective of document (5) is to ensure the elongation of the probe **up to the mutation site** and the insertion at this site of a nucleotide derivative which protects the probe from subsequent digestion with eg an exonuclease. From this teaching, alone or in combination with that of documents (6) or (9), the skilled person would not have readily derived the idea of a method based on the achievement or prevention of selective primer extension beyond the mutation site of either a sequence containing a suspected variant nucleotide or the corresponding sequence containing the normal nucleotide.

14. It is true that the skilled person knew from the art that for the polymerisation step to occur pairing has to be present between the primer terminus and the template (cf documents (2) and (3)) and that, in case of a mismatch, several polymerases would exert a proofreading function (cf document (2)). However, this prior art, alone or in combination with documents (9) or (6), would not have suggested using a polymerase lacking the proofreading activity in order to detect a

nucleotide variation in a target sequence based on the elongation or non-elongation of a specifically designed primer.

15. The skilled person was aware of the availability of various polymerases, among them of the thermostable Taq polymerase of document (21) which was used therein to improve the amplification step in the framework of the analysis of nucleotide sequences. However, nothing also in the latter document indicated to the skilled person the possibility of taking advantage of its lack of proofreading activity in a detection method based on selective primer extension.

16. For these reasons, in the board's judgement the method according to claims 1 to 9 at issue involves an inventive step.

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 the main request as submitted in the oral proceedings, description as granted except for pages 2, 6, 7, 10 and 11 as submitted in the oral proceedings and drawings as granted.

The Registrar: The Chairman:

U. Bultmann L. Galligani