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Datasheet for the decision of 22 March 2007

Case Number: T 0364/06 - 3.3.04

Application Number: 89310424.0

Publication Number: 0364255

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Multiplex genomic DNA amplification for deletion detection

Patentee:
BAYLOR COLLEGE OF MEDICINE

Opponent:
-

Headword:
Multiplex DNA amplification/BAYLOR

Relevant legal provisions:
EPC Art. 83, 111(1), 123(2)

Keyword:
"Added subject-matter - (no)"
"Sufficiency of disclosure - (yes)"
"Remittal - (yes)"

Decisions cited:
T 0014/83, T 0019/90, T 0792/00, T 1091/00

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0364/06 - 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 22 March 2007

Appellant: (Patent Proprietor)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 17 January 2006 revoking European patent No. 0364255 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chair: G. Alt
Members: M. Wieser
R. Moufang

Summary of Facts and Submissions

I. The appeal was lodged by the Patent Proprietor (Appellant) against the decision of the Opposition Division, whereby the European patent No. 0 364 255 was revoked pursuant to Article 102(1) EPC.

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

All four Opponents withdrew their oppositions during the opposition procedure and are therefore no longer parties to the procedure with regard to substantive issues.

III. The Opposition Division had decided that the main request, claims 1 to 8 as granted, and auxiliary request 1 before them did not meet the requirements of Article 83 EPC. Moreover, they decided that the claims of auxiliary requests 2 and 3 before them did not meet the requirements of Article 123(2) EPC.

IV. The Board expressed its preliminary opinion in a communication dated 18 September 2006.

Oral proceedings were held on 22 March 2007.

V. The Appellant requested that the decision under appeal be set aside and the case be remitted to the department of first instance for further prosecution on the basis of claims 1 to 7 as granted.

VI. Claim 1 as granted read as follows:

"A method for simultaneously detecting in a sample target DNA sequences, comprising the steps of:

providing in a common reaction vessel the sample in single-stranded form and pairs of oligonucleotide primers, each pair specific for a different sequence, one primer of each pair substantially complementary to a part of the sequence in the sense-strand and the other primer of each pair substantially complementary to a different part of the same sequence in the complementary anti-sense strand;

annealing the pairs of primers to their complementary sequences;

simultaneously extending said pairs of annealed primers from each primer's 3' terminus to synthesize an extension product complementary to the strands annealed to each primer, said extension products, after separation from their complement, serving as templates for the synthesis of an extension product from the other primer of each pair;

separating said extension products from said templates to produce single-stranded molecules of the target sequences;

amplifying said single stranded target sequences by repeating, at least once, said annealing, extending and separating steps; and

identifying whether said amplified extension products have been synthesised from each different sequence, as a measure of the presence or absence of each target sequence, characterised in that:

the method is adapted to detect simultaneously more than two target sequences by utilising more than two pairs of oligonucleotide primers."

Dependent claims 2 to 7 referred to preferred embodiments of the method according to claim 1.

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

(1) Biochim. Biophys. Acta; vol.949, January 1988, pages 43 to 48

(2) Proc. Natl. Acad. Sci. USA; vol.85, September 1988, pages 6977 to 6981

(5) N. Engl. J. Med.; vol.317, 1987, pages 985 to 990

(6) Nature; vol.329, 1987, pages 293 to 294

(10) Nucleic Acids Research; vol.16, September 1988, pages 8233 to 8243

(12) Science; vol.239, January 1988, pages 487 to 491

(21) Nature; vol.333, June 1988, pages 858 to 860

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

Successful working of multiplex PCR required the adaptation of three critical parameters, namely increase of PCR elongation time, increase of the amount of Taq polymerase and balancing of the melting temperatures of all primer pairs. The patent in suit contained workable experiments showing the successful simultaneous amplification of up to seven separate DNA sequences. In paragraphs [0045] and [0046] on pages 6 and 7, the patent contained sufficient general information that would have enabled the skilled reader to identify the critical parameters and to realize the way in which they had to be adapted in order to achieve the desired technical effect, namely the simultaneous amplification of more than two target sequences by utilising more than two primer pairs. The fact that the patent as granted also contained an erroneous example 8, (lacking any experimental data to substantiate the alleged result), did not result in a lack of sufficient disclosure (Article 83 EPC).

Reasons for the decision

Amendments - Article 123(2) EPC

1. The Opposition Division has decided in point (3) on pages 3 to 8 of the decision under appeal, that the claims as granted met the requirements of Article 123(2) EPC.

The Board, having carefully considered all objections brought forward by the former Opponents and dealt with by the Opposition Division sees no reason to deviate from this decision.

Thus, claims 1 to 7 as granted do not contain subject-matter extending beyond the content of the application as filed and meet the requirements of Article 123(2) EPC.

Sufficiency of disclosure - Article 83 EPC

2. The subject-matter of claim 1 is a method for simultaneously detecting target DNA sequences by applying a technique known as Polymerase Chain Reaction (PCR). The method is characterised in that it is **adapted** to detect simultaneously more than two target sequences by utilising more than two pairs of oligonucleotide primers. This method is also designated multiplex PCR.

The relevant state of the art is acknowledged on page 2 and 3 of the patent in suit. The prior art documents mentioned and discussed there are said to disclose methods which are able to amplify only one or two target sequences. Multiple sequences can be amplified sequentially only. The presently claimed method is said to be an improvement on the PCR method which solves the problems encountered when primers for multiple sequences are reacted simultaneously (page 2, lines 38 to 41).

3. The Board has no reason to doubt the Appellant's submissions saying that the **adaptation** of the PCR technique, which is required to achieve the desired technical effect, namely simultaneous amplification of more than two target sequences, concerns the following three parameters:

The **PCR elongation time** and the **amount of Taq polymerase**, which both have to be increased compared to PCR wherein one or two target sequences are amplified, and the **primer design**, which has to be chosen such that all primers have balanced melting temperatures (T_m) (see Appellant's letter dated 26 May 2006, page 10).

These three parameters are not mentioned in claim 1.

4. According to Article 83 EPC a European patent application (or a European patent) must disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

It has been consistent case law of the Boards of Appeal since at least T 14/83 (OJ EPO 1984, 105) that sufficiency of disclosure within the meaning of Article 83 EPC must be assessed on the basis of the application as a whole - including the description and claims - (see Case Law of the Boards of Appeal of the EPO, 5th Edition 2006, Chapter II.A.1).

5. When examining this issue, namely if the patent specification as a whole meets the requirements of Article 83 EPC, the Board must be satisfied firstly, that the patent specification places the skilled person in possession of at least one way of putting the

claimed invention into practice, and secondly that the skilled person can put the invention into practice over the whole scope of the claim (decision T 792/00 of 2 July 2002).

6. Example 1 on pages 6 and 7 of the patent in suit describes the working conditions of multiplex PCR. Example 2 discloses in table 1 on page 8 seven primer pairs specific for seven exons of the human Duchenne muscular dystrophy (DMD) gene.

Example 4 on page 16, describes the use of six of these primer pairs for the simultaneous detection of six human DMD gene exons. The results are shown in Figures 3A and 3B. Example 5 discloses multiplex DNA amplification for prenatal diagnosis of DMD (results are shown in figure 4). Example 6 is concerned with prenatal diagnosis, using multiplex DNA amplification of chorionic villus specimen (CVS) DNA (results in figure 5). Example 7, on page 16, lines 51 to 57, discloses the simultaneous amplification by multiplex PCR of seven human DMD gene exons. The primer sets used were those shown in table 1. The results of the successful multiplex PCR are shown in figure 6 on page 33 of the patent.

Examples 4 to 7 explicitly refer to the working conditions described in example 1.

In the light of this disclosure, the Board is satisfied that the first requirement, mentioned in point (5) above, namely that the patent specification must place the skilled person in possession of at least one way of

putting the claimed invention into practice, is met by the patent in suit.

7. When asking if the patent specification places a skilled person in the position to carry out the invention over the whole scope claimed, it has to be examined if the patent specification as a whole contains sufficient information for the skilled reader to conclude that the simultaneous detection of more than two arbitrary target sequences by multiplex PCR requires the adaptation of the three critical process parameters indicated in point (3) above.

8. The Opposition Division, in the decision under appeal, came to the conclusion that the patent specification contained sufficient information to instruct a skilled reader to increase the PCR elongation time and the amount of Taq polymerase. However, they took the view that the disclosure of the specification was insufficient with regard to the necessary primer design. The skilled person would not have concluded that the primers had to be designed such that they all had balanced melting temperatures. Therefore, in the view of the Opposition Division, the requirements of Article 83 EPC were not met.

9. Page 7, lines 2 to 3, of the patent specification, which form part of example 1, describing the conditions for multiplex PCR, read:

"Thus, as the number of amplified sequences increase and/or the length of amplified sequences increases, the time must be increased".

Lines 20 to 22 on the same page disclose the temperature duration applied during the reaction cycles. The PCR elongation time at 65° C is said to continue 3,5 minutes.

Thus, the patent specification explicitly refers to the critical parameter "**PCR elongation time**" and indicates the way in which it has to be adapted. The example given, namely 3,5 minutes, is significantly increased compared to times usually applied in PCR (see for instance document (6), page 293, figure 1, which discloses a PCR elongation time of 45 seconds).

10. Page 7, line 14 of the patent specification reads:

"The enzyme, Taq polymerase, was added to achieve a final concentration of 100 units/mL."

This sentence refers to the critical parameter **"amount of Taq polymerase"**, but does not, in itself, contain an invitation to the skilled reader to adapt it in a way so that it is increased compared to the amount of enzyme used in conventional PCR.

However, the working conditions used for PCR are disclosed in a large number of prior art documents, like for instance in document (6), which discloses a Taq polymerase concentration of 2 to 3 units/100 µl (page 293, figure 1), and in document (10) disclosing 1 unit/100 µl (passage bridging pages 8234 and 8235). Thus, the skilled person, being familiar with the disclosure in the relevant prior art, will realize that the amount of Taq polymerase used in the presently

claimed method is increased with regard to the amount used in conventional PCR.

11. Page 7, lines 3 to 8 of the patent specification read:

"The temperature is dependent on the length, the uniqueness of the primer sequence and the relative percentage of GC bases. The longer the primers, the higher the temperature needed. The more unique the sequence, the lower the temperature needed to amplify. GC rich primers need higher temperatures to prevent cross hybridization and to allow unique amplification. However, as the AT percentage increases, higher temperatures cause these primers to melt. Thus, these primers must be lengthened for the reaction to work."

This passage is concerned with PCR reaction temperature. It is said that the reaction temperature, to a certain degree, depends on the **primer design**, namely length, uniqueness and sequence of the used primers. In the last two sentences the skilled reader is told that the tendency of primers to melt rises when their AT percentage rises. Thus, primers with high AT percentage have a lower T_m, which is defined as the temperature at which half of the primer binding sites at the DNA template are occupied. The skilled reader is instructed that the design of such primers has to be adapted. For the reaction, namely multiplex PCR, to work, these primers have to be lengthened, with the effect that their T_m is increased.

12. The primer pairs used in examples 4 and 7 for the simultaneous amplification and detection of six, respectively seven, exons of the human DMD gene are

shown in table 1 on page 8 of the patent specification. This table does not indicate the T_m values of the primers.

However, the Appellant has provided evidence showing that the primers of table 1 cover melting temperatures (T_m) of 70 to 76°C. Thus, the difference between the primer having the lowest T_m and the primer having the highest T_m is only 6°C. The Appellant has moreover examined the range of T_m covered by the primers used in prior art documents (1), (2), (5), (6), (12) and (21) and has found that they vary from 12°C (document (1)) to 46°C (document (2)); (see Appellant's letter dated 12 August 2005, page 11).

13. The skilled person in the field of PCR is aware that T_m of the used primers is an important parameter, which he/she can determine by a simple mathematical calculation and which he/she has to know in order to design the reaction conditions.

14. Thus, to summarise, the patent specification discloses on page 7 that, for multiplex PCR to work, it is important that primers whose melting temperature is too low are adapted. They have to be lengthened so that their melting temperature is raised. The specification contains specific examples using fourteen primers whose melting temperature cover a range which is significantly smaller than the range covered by the primers according to various prior art documents. The determination of the melting temperature of primers used for PCR belongs to the essential routine activities of a person skilled in the field of DNA

amplification and detection and can be carried by a simple mathematical calculation.

15. In the light of the disclosure on page 7 and in table 1 on page 8, the Board is convinced that the skilled person reading the patent specification would have identified the three critical parameters (PCR elongation time, amount of Taq polymerase and primer design to achieve balanced T_m) in order to successfully operate multiplex PCR, and would have adapted them compared to the values applied in conventional PCR.

Thus, the Board is satisfied that the skilled person could put the invention, according to claims 1 to 7 as granted, into practice over the whole scope of the claims.

16. The Opposition Division has argued that the patent specification also contained example 8, which referred to multiplex DNA amplification for the simultaneous detection of mutations leading to multiple common genetic diseases. This example used, in addition to the primers shown in table 1, three primer pairs, designated (A), (B) and (C), with unbalanced T_m. These primer pairs were the subject of claim 8 of the patent as granted, which formed part of the main request and of auxiliary request 1 before the Opposition Division.

The Opposition Division concluded that example 8, which they considered to be described as being part of the invention, taught the skilled reader that balancing T_m of the primers was not a requirement for carrying out multiplex PCR and that accordingly in this respect the

disclosure of the patent specification was not sufficient.

17. Example 8 differs from examples 4 to 7 in so far as it is the only example not referring to the conditions described in example 1. Moreover, the patent specification does not contain any results of example 8 in the form of gel electrophoresis data, as it is provided for examples 4 to 7 (see figures 3 to 6).

18. Claim 8 of the patent as granted, referring to the unbalanced primer pairs used in example 8, is not part of Appellant's actual request.

The Board has found in points (6) and (15) above that the patent specification places the skilled person in possession of at least one way of putting the invention according to claims 1 to 7 as granted into practice, and that the skilled person could put the invention into practice over the whole scope of the claims.

Thus, the existence of example 8 in the patent as granted does not result in a problem of lack of sufficient disclosure under Article 83 EPC.

However, in case the description is not adapted, a problem may arise under Article 84 EPC as claims 1 to 7 as granted do not seem to be supported by example 8. This problem will have to be considered within the course of the further proceedings of the case before the department of first instance (see points (21) to (23) below).

19. The Board finally notes that claim 1, as a result of its wording ("... the method is adapted to ..."), theoretically also embraces methods for multiplex PCR not relying on the control and monitoring of the three critical parameters, elongation time, amount of Taq polymerase and primer design.

However, the Board is not aware of any evidence showing that a method neglecting the importance of these parameters would lead to the desired result, namely the simultaneous amplification of more than two target sequences.

A patent may only be objected to for lack of sufficient disclosure if there are serious doubts, substantiated by verifiable facts. The mere fact that a claim is broad is not in itself a ground for considering the patent as not complying with the requirements of sufficient disclosure under Article 83 EPC (cf decision T 19/90, OJ EPO 1990, 476).

20. The Board arrives at the decision that the patent discloses the invention according to claims 1 to 7 as granted in a manner sufficiently clear and complete for it to be carried by a person skilled in the art. The requirements of Article 83 EPC are met.

Remittal to the department of first instance
Article 111(1) EPC

21. According to Article 111(1) EPC the Board of Appeal may either exercise any power within the competence of the department which was responsible for the decision

appealed or remit the case to the department for further prosecution.

Remittal to the department of first instance is at the discretion of the board. Although Article 111(1) EPC does not guarantee an absolute right to have all the issues in the case considered by two instances, it is well recognised that any party should preferably be given the opportunity to have two readings of the important elements of the case (cf decision T 1091/00, 2 July 2002).

The essential function of appeal proceedings is to consider whether the decision which has been issued by the first instance department is correct. Hence, a case is normally remitted, if essential questions regarding the patentability of the claimed subject-matter have not yet been examined and decided by the department of first instance.

In particular, remittal is taken into consideration by the Boards in cases where a first instance department issues a decision solely upon one particular issue which is decisive for the case against a party and leaves other essential issues outstanding. If, following appeal proceedings, the appeal on the particular issue is allowed, the case is normally remitted to the first instance department for consideration of the undecided issues.

22. The Opposition Division in the decision under appeal has only dealt with the question of sufficiency of disclosure, without comprehensively touching any other substantial requirements of the EPC.

Thus, fundamental requirements for the maintenance of a patent, like novelty (Article 54 EPC) and inventive step (Article 56 EPC), have not yet been examined by the department of first instance.

23. In the light of the specific situation in the present case - all four original Opponents have withdrawn their oppositions and the Patent proprietor remained the only party to the procedure - the Board, although being aware that this could lead to a further delay of the procedure, considers it to be justified and appropriate to allow claims 1 to 7 as granted to be examined by two instances and decides therefore, at its discretion under Article 111(1) EPC, to remit the case to the department of first instance for further prosecution.

Order

For these reasons it is decided:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance for further prosecution on the basis of claims 1 to 7 as granted.

Registrar: Chair:

P. Cremona G. Alt