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D E C I S I O N
of 3 May 1999
& 26 August 1999

Case Number: T 0965/98 - 3.3.4

Application Number: 92201243.0

Publication Number: 0509612

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Process for amplifying and detecting nucleic acid sequences

Applicant:
F. Hoffmann-La Roche AG

Opponent:
-

Headword:
DNA amplification/HOFFMANN-LA ROCHE AG

Relevant legal provisions:
EPC Art. 84, 54
EPC R. 88, 89

Keyword:
"Clarity (yes)"
"Novelty (yes)"
"Inventive step (yes)"
"Correction of obvious mistakes (allowable)"

Decisions cited:
-

Catchword:

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Case Number: T 0965/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 3 May 1999 & 26 August 1999

Appellant: F. Hoffmann-La Roche AG
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 22 April 1998 refusing European patent application No. 92 201 243.0 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
S. C. Perryman

Summary of Facts and Submissions

I. European patent application No. 92 201 243.0, publication No. 0 509 612, with the title: "Process for amplifying and detecting nucleic acid sequences" was filed as a divisional application to the application published under No. 0 200 362. It was refused by the Examining Division in a decision dated 22 April 1998.

II. The decision of the Examining Division was taken on the basis of the request filed on 31 January 1997.

Claim 1 of this request read as follows:

"1. A first and a second single-stranded oligonucleotide allowing amplification of a specific nucleic acid sequence contained in a single- or double-stranded nucleic acid or in a mixture of such nucleic acids, wherein

(a) one oligonucleotide of said oligonucleotides is substantially complementary to said single-stranded nucleic acid or to one strand of said double-stranded nucleic acid;

(b) the other oligonucleotide of said oligonucleotides is substantially complementary to a complement of said single-stranded nucleic acid or to the other strand of said double-stranded nucleic acid;

(c) said oligonucleotides additionally contain on the 5' end a sequence which is non-complementary to said nucleic acid, said sequence comprising a restriction site; and wherein

(d) the parts of said oligonucleotides that have substantial complementarity are different and define the termini of the specific nucleic acid sequence to be amplified."

Dependent claims 2 to 6 related to further features of the claimed oligonucleotides and dependent claims 7 to 11 related to various uses thereof.

The Examining Division found that the subject-matter of claim 1 was unclear as the claimed oligonucleotides were characterised by their complementarity to a template which remained unspecified. Such a wording made it impossible for the skilled person to know whether he or she was working inside or outside of the scope of the claim.

Because of this unclarity in wording, the subject-matter of claim 1 encompassed oligonucleotides as described in document (5) or (6) and, thus, lacked novelty.

III. The Appellants lodged an appeal against this decision, paid the appeal fee and submitted a statement of grounds for the appeal.

IV. The following documents are mentioned in the present decision:

(4): EP- A O 090 433

(5): Wallace, R. et al., Gene, Vol. 16, pages 21 to 26, 1981,

(6): New England Biolabs 1983/1984 Catalog, page 33.

V. At oral proceedings, the Appellants submitted a new main request for consideration by the Board.

Claim 1 read as follows:

"1. A first and second single-stranded oligonucleotide allowing amplification of a specific **template** nucleic acid sequence contained in a single- or double-stranded nucleic acid or in a mixture of such nucleic acids, wherein

(a) one oligonucleotide of said oligonucleotides **contains** a part which is substantially complementary to said **template** nucleic acid **sequence in said single-stranded nucleic acid** or **in** one strand of said double-stranded nucleic acid;

(b) the other oligonucleotide of said oligonucleotides **contains a part** which is substantially complementary to said **template nucleic acid sequence** in **said single-stranded nucleic acid** or in the other strand of said double-stranded nucleic acid;

(c) said parts of oligonucleotides (a) and (b) have attached to their 5'-end a nucleotide sequence which is non-complementary to said template nucleic acid sequence and which comprises a restriction site; and wherein

(d) the parts of said oligonucleotides of (a) and (b) that have substantial complementarity are different and define the termini of the specific template nucleic acid sequence to be amplified." (Emphasis of amendments by the board).

Claims 2 to 11 remained unchanged.

VI. The Appellants argued essentially as follows:

Clarity:

To restrict the claimed oligonucleotides to those that could be annealed to a specifically mentioned template would amount to an unreasonable restriction of the scope of protection, seeing that the amplification could be carried out with any template.

The concern of the Examining Division that the skilled person would not know, when working with two oligonucleotides whether he/she was working inside or outside of the scope of the claim was unfounded. Indeed a situation where two oligonucleotides would happen to hybridize by chance to the extremities of a "template" DNA molecule although their sequences had not been derived from that of said DNA molecule was theoretically conceivable, but had no likelihood to occur.

The skilled person reading claim 1 would have no difficulty in understanding which two oligonucleotides would be of use to amplify which specific template. The claim was clear.

Novelty and inventive step

Both documents (5) and (6) disclosed primers which were fully complementary to the sequence to be amplified and contained a restriction site, rather than primers which had a DNA fragment carrying a restriction site attached to their 5' end. Novelty was not at stake.

Document (4) could be taken as closest prior art. It disclosed a process for isolation of modified DNA sequences whereby the DNA to be modified was present on a single-stranded DNA vector, annealed to a specific primer carrying the required modification, and if desired, to an additional piece of DNA carrying a restriction site as marker. The hybrid composed of the single-stranded vector and of the oligonucleotide primer was made double-stranded in vitro and said double-stranded molecule was amplified in vivo.

The objective technical problem to be solved by the present application was that of providing means for the specific and precise amplification of a given template.

It was doubtful whether this problem could be derived from document (4) as this document was concerned with site-directed mutagenesis rather than with DNA amplification. Furthermore, the structure of the primers described in document (4) was not such as to suggest the structure of the primers which solved the objective technical problem. The subject-matter of claim 1 was inventive.

VII. At the oral proceedings, the Appellants requested that the decision under appeal be set aside and that a

patent be granted on the basis of claims 1 to 11 submitted at the oral proceedings on 3 May 1999.

VIII. After deliberation by the Board the following decision was announced by the Chairwoman:

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 11 submitted at the oral proceedings on 3 May 1999 and a description to be adapted.

IX. During preparation of the reasons for the above decision, the Board queried with the Appellants whether the wording of claim 1 submitted at oral proceedings on 3 May correctly reflected their intentions.

X. On 19 August 1999, the Appellants submitted a request pursuant to Rule 88 EPC for correction and an amended set of claims 1 to 11. The only change compared to the claims 1 to 11 submitted at the oral proceedings on 3 May was that amended claim 1(b) read:

(b) the other oligonucleotide of said oligonucleotides contains a part which is substantially complementary to *a complement of* said template nucleic acid sequence in said single-stranded nucleic acid or in **the other** *said* strand of said double-stranded nucleic acid;

(Additions compared to the claim submitted on 3 May 1999 in italics, deletions in bold.)

In support for this request for correction of an obvious error, the Appellants submitted that:

- Support for the amended wording could be found e.g. in claim 1 of the application as originally filed, in particular feature (a) requiring that the elongation product synthesized from one primer, when it is separated from its complement, serves as a template for synthesis of an extension product of the other primer.

- It was apparent that, in the first embodiment, the oligonucleotide of claim 1(a) had to be complementary to a template nucleic acid sequence in a single-stranded nucleic acid and accordingly, the oligonucleotide of claim 1(b) had to be complementary to a **complement** of the template nucleic acid sequence in said single-stranded nucleic acid to which the oligonucleotide of claim 1(a) hybridized.

- Likewise, in the second embodiment, the oligonucleotide of claim 1(a) that was complementary to a complement of said template nucleic acid sequence in one strand of said double-stranded nucleic acid required that the corresponding oligonucleotide of claim 1(b) be complementary to a complement of said template nucleic acid sequence in said strand of said double-stranded nucleic acid.

- Otherwise amplification of the template nucleic acid would not be possible.

XI. Having considered the request for correction pursuant to Rule 88 EPC, the Board decided on 26 August 1999 to allow the request and pursuant Rule 89 EPC to amend its decision as announced at the oral proceedings on 3 May 1999 to refer to claims 1 to 11 submitted on 19 August 1999 instead of those claims as submitted on 3 May 1999.

Reasons for the Decision

1. The appeal is admissible

Request for amendment pursuant to Rule 88 EPC

2. For the reasons given by the Appellants (see point X. above) the Board agrees that it is immediately evident that the corrected version of Claim 1(b) submitted on 19 August 1999 is what was intended to be submitted at the oral proceedings on 3 May and that nothing else would have been consistent with the arguments then submitted. Accordingly, the Board grants the request under Rule 88 EPC. In the following, the set of claims in the form submitted on 19 August 1999 are referred to.

Article 123(2) EPC, added subject-matter:

3. The subject-matter of claim 1 finds support on pages 22 and 23 of the patent application as filed. The requirements of Article 123(2) EPC are fulfilled.

Article 84 EPC: clarity

4. The reasoning of the Examining Division on Article 84 EPC (see section II above), while correct in theory, does not take into account how the sequences of oligonucleotides such as claimed, which are complementary to a specific template are obtained. It is readily apparent from the state of the art cited in the present case, that the sequence of these oligonucleotides is derived from the **known** sequence of the template: in document (4), it is the known sequence of insulin which serves to devise the mutated primers which will help in the isolation of modified versions of the insulin gene; in document (5), the primers are synthesized starting from the known sequence of the pBR322 plasmid; in document (6) the primer sequences are derived from the known sequence of M13.

5. In the Board's judgment, these examples reflect the way in which oligonucleotides complementary to a specific template are in general obtained. The probability that two oligonucleotides would by chance be substantially complementary to the termini of an unidentified template from which their sequences are not derived can be ignored as de minimis.

6. Thus, claim 1 relates to a kit comprising two oligonucleotides, the sequences of which are derived from the sequence of the termini of a specific albeit undefined template. The skilled person would have no difficulty in determining which oligonucleotides fell under the scope of the claim. The requirements of Article 84 EPC are fulfilled.

Article 54 EPC, novelty:

7. Documents (5) and (6) were cited as novelty destroying for the subject-matter of claim 1. Both of them disclose oligonucleotide primers for DNA sequencing. These oligonucleotides (document (5), Table 1, document (6), page 33) contain a restriction site within their sequence. They are, thus, different from the claimed oligonucleotides. No other documents on file disclose an oligonucleotide primer carrying at its 5'end a DNA sequence which is non-complementary to the template and contains a restriction site. Novelty is acknowledged.

8. Inventive step and sufficiency of disclosure were not dealt with in the decision of the Examining Division. As the Appellants are here desirous of avoiding the lengthening of the procedure which would necessarily result from remitting the case back to the first instance and, in particular, because all the necessary information would appear to be before the Board, the Board in this case exercises the discretion it has under Article 111(1) EPC, to consider itself whether these two requirements of the EPC are fulfilled.

Sufficiency of disclosure

9. The description of the patent application, Example 2, part 1 gives the necessary information for the synthesis and characterisation of oligonucleotides complementary to a specific template. Obtaining such oligonucleotides attached to a DNA fragment carrying a restriction site would have been a matter of routine at the priority date. The specification also provides numerous examples of the ability of the oligonucleotides to allow amplification of a specific

template. The requirements of Article 83 EPC are fulfilled.

Inventive step

10. Document (4) is considered to be the closest prior art. It is concerned with the **in vivo** isolation of modified proinsulin precursor DNA sequences. In a first step, the insulin coding sequence is obtained in single-stranded form by cloning it into an appropriate vector. Two oligonucleotides are then hybridized to this single stranded template: one of them contains the modified nucleotide to be introduced in the insulin coding sequence and the other is slightly altered compared to the corresponding sequence on the template, so that it carries a restriction site. The positions where these primers hybridize to the insulin coding sequence are solely dependent on the position of the sequence which is to be modified and on the position where it is feasible to introduce a restriction site, respectively. After hybridisation, the primers are extended and ligated to form together with the single-stranded template vector, a circular double-stranded vector which is transformed in E.coli. Upon replication, a group of vectors is, thus, obtained which comprises the modified proinsulin coding sequence in double-stranded form. Further replication in E.coli leads to their amplification. They are detected by their sensitivity to the restriction enzyme recognizing the restriction site which was present on the second primer.
Document (4) can be regarded as disclosing a first and second oligonucleotides which enable the in vivo amplification of proinsulin encoding DNA.

11. Starting from this closest prior art, the technical problem to be solved may be defined as providing means for the **in vitro** amplification of a DNA template.

12. The solution provided by the patent application is the oligonucleotides of claim 1 with the following characteristics:

(a) they do not hybridize to the same strand of the template,

(b) they define the termini of the template, and

(c) each of them is attached to a third oligonucleotide which contains a restriction site.

13. In the Board's opinion, the structure of the DNA primers disclosed in document (4) does not in any way suggest the structure of the claimed oligonucleotides. This is particularly evident in relation to characteristics (a) and (b) (compare points 10 and 12 above) but is also true of characteristic (c): the restriction site in the oligonucleotide of document (4), which could not be of use for the cloning of the amplified sequence since it is located within this sequence, does not render obvious adding a restriction site to the extremities of the claimed oligonucleotides for subsequent cloning purposes.

14. Documents (5) and (6) which were discussed in the novelty issue are of such a different technical character that one cannot assume that the skilled person would combine them with document (4) to arrive in an obvious way at the claimed subject-matter.

Inventive step is acknowledged.

Correction of the originally announced decision pursuant to Rule 89 EPC

15. As the Board has considered the claim request as modified pursuant to the request under Rule 88 EPC, and as this request has been found allowable, the Board also exercises its power under Rule 89 EPC to correct the decision as announced on 3 May 1999 to refer to the claims 1 to 11 filed on 19 rather than to claims 1 to 11 filed at the oral proceedings on 3 May 1999.

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 11 submitted on 19 August 1999 and a description to be adapted.

The Registrar: The Chairwoman:

U. Bultmann U. Kinkeldey