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D E C I S I O N
of 2 June 2003

Case Number: T 0753/00 - 3.3.4

Application Number: 91914066.5

Publication Number: 0542810

IPC: C07K 15/00

Language of the proceedings: EN

Title of invention:
Methods for the production of proteins with a desired function

Applicant:
B.R. CENTRE LIMITED, et al

Opponent:
-

Headword:
Production of proteins/B.R. CENTRE LIMITED, et al

Relevant legal provisions:
EPC Art. 54, 56, 123(2)

Keyword:
"Main request - inventive step (no)"
"Auxiliary requests - allowability of amendments (no)"

Decisions cited:
T 0207/94, T 0939/92, T 0187/91, T 1067/97, T 0386/94, T 0923/92, T 0333/97

Catchword:
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Case Number: T 0753/00 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 2 June 2003

Appellant: B.R. CENTRE LIMITED
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Decision under appeal: Decision of the Examining Division of the European Patent Office posted 17 December 1999 refusing European patent application No. 91 914 066.5 pursuant to Article 97(1) EPC.

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: M. R. J. Wieser
S. U. Hoffmann

Summary of Facts and Submissions

I. The patent application EP 91 914 066.5, publication number EP-A-0 542 810, (international publication number WO-A-92/02 551), with title "Methods for the production of proteins with a desired function", was refused by the examining division under Article 97(1) EPC, as it was found that the claims of the request then on file did not meet the requirements of Article 56 EPC.

In its decision the examining division also gave reasons that the application did not meet the requirements of Articles 83 and 123(2) EPC.

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 37, filed at the oral proceedings, or on the basis of the first auxiliary request, claim 1 to 38, filed 23 July 1997, or on the basis of the second auxiliary request, claims 1 to 37, filed 20 May 2003. Moreover, the appellants requested the refund of the appeal fee arguing a substantial procedural violation.

III. Claim 1 of the main request, filed at oral proceedings on 2 June 2003, read as follows:

"A method for producing a protein with a desired function, comprising:

(a) providing from a warm-blooded animal a population of antibody-forming B cells or progeny thereof suspected of containing at least one cell that produces an antibody exhibiting the desired function;

(b) suspending the population of antibody-forming cells in a medium, the medium having an indicator system incorporated therein, said indicator system being capable of indicating the presence and location of a cell which forms antibodies exhibiting the desired function;

(c) identifying a cell forming an antibody exhibiting the desired function;

(d) isolating the identified antibody-forming cell from the medium;

(e) recovering RNA from said isolated antibody-forming cell;

(f) generating cDNA from said RNA; and

(g) exponentially amplifying DNA of the variable region or a portion thereof which confers the desired function of the antibody produced by the isolated antibody-forming cell by performing sequential reactions with a DNA polymerase;

(h) determining the amino acid sequence of the variable region or a portion thereof which confers the desired function of the antibody produced by the isolated antibody-forming cell; and

(i) synthesizing a protein with a desired function, said protein containing the amino acid sequence of said variable region or portion thereof which confers the desired function."

IV. Claim 1 of both auxiliary requests differed therefrom only in point (g), which read:

"(g) amplifying DNA of the variable region or a portion thereof which confers the desired function of the antibody produced by the isolated antibody-forming cell utilizing a nested primer;".

V. The following document is referred to in this decision:

(5) BIO/TECHNOLOGY, Vol. 7, September 1989, pages 934 to 938

VI. The arguments of the appellants may be summarised as follows:

The claimed method was novel and not obvious with regard to the disclosure in document (5), which was concerned with cloning of human monoclonal antibody variable region genes from hybridoma cells only. All statements in said document referring to the use of B cells were considered as being mere guesswork, not substantiated by experimental evidence. A skilled person reading document (5) would have been faced with a prejudice in the art and would not have had a reasonable expectation of success when following the speculations disclosed in the document in order to solve the problem underlying the present application. Document (5) did not disclose exponential amplification of DNA by sequential reactions with a DNA polymerase.

The reference to nested primers in claim 1 of both auxiliary requests was based on the application as originally filed, in accordance with the requirements of Article 123(2) EPC.

Reasons for the Decision

The Main Request

1. The claims are based on the application as originally filed. Claim 1 is a combination of originally filed claims 1 and 4, having additional basis in claim 15 ("warm-blooded animal"), page 6, lines 23 to 24 ("antibody forming B cells or progeny thereof") and page 25, lines 11 to 14 ("exponentially amplifying DNA ... by performing sequential reactions with a DNA polymerase").

   The requirements of Article 123(2) EPC are met.

2. The method according to claim 1 is not disclosed in any of the prior art documents on file. Document (5) speculates that the method disclosed therin using hybridoma cells may also be applied to single B-cells.

   Article 54(2) EPC requires that the state of the art in order to anticipate a claim has to make publically available the subject-matter of the claim *in toto*. This means in the present case, which refers to the complex technical field of recombinant DNA technology, that a skilled person must be provided with technical teaching demonstrating the actual realisation of announced experiments.

The board concludes therefore that the subject-matter of claim 1 is not anticipated by the disclosure in document (5) and is novel within the meaning of Article 54 EPC.

3. The closest prior art, document (5), discloses cloning of human monoclonal antibody variable region genes from single hybridoma cells by polymerase chain reaction (PCR) using mixed primers.

4. The document describes a general approach to rapidly obtain the DNA sequence encoding the variable region of any immunoglobulin chain using PCR and a mixture of upstream primers corresponding to the leader sequence, and one downstream primer designed from the conserved nucleotide sequence of the constant region. The mixture of oligonucleotides used for priming upstream of sequences encoding the immunoglobulin variable region was designed from data for a number of previously determined leader sequences. The known heavy chain leader sequences were grouped into three major groups. Similarly the κ and λ light chain upstream primers where derived from different known sequences. By using mixed sets of primers it was demonstrated that PCR can be used as a general approach to obtain DNA encoding immunoglobulin variable regions, despite the fact that the 5' end is variable and no consensus primer sequence can be designed (pages 934 to 935).

5. In the experimental part starting on page 936, document (5) reports of experiments with five different hybridoma cell lines. RNA is prepared from the hybridomas and reverse transcribed into cDNA. On page 937, left column, the PCR conditions are described (buffer concentrations, temperature profile, number of

cycles) and the used primers are shown in table 1. Finally, DNA sequencing of the purified PCR products is described. Figure 5 shows results from PCR amplification of heavy chain variable regions starting with each of 1, 10, 100, 10 000 and 100 000 cells.

6. In the light of this closest prior art, the problem to be solved by the present application is defined as the production of antibodies with desired variable regions not using hybridomas.

7. At the oral proceedings the appellants argued that the subject-matter of claim 1 differs from the disclosure in document (5) in point (g) of it which speaks of **"exponentially amplifying DNA ... by performing sequential reactions with DNA polymerase;"** (emphasis added by the board). This was seen in contrast to the disclosure on page 937 of document (5), which refers to PCR amplification being run in a conventional cycler set for 20 to 60 cycles, each consisting of the three sequential working steps denaturation of double stranded DNA, primer annealing and primer extension. Appellants argued that the term "sequential reactions with DNA polymerase" in claim 1(g) is understood by the skilled person as meaning that sequential PCR cycles were performed by use of different, nested primers.

8. The board does not agree to this interpretation. The application as originally filed does not provide the skilled person with a basis to allow him to give the cited passage of claim 1(g) this interpretation. The PCR technique is described on page 25, lines 4 to 29 of the description as being well known in the art. Reference is made to various patents and text books describing it. Lines 11 to 14 read: *"Briefly, cDNA*

segments encoding the portion of the antibody that confers the desired function are exponentially amplified by performing sequential reactions with a DNA polymerase." The board is convinced that this sentence is understood as referring to conventional PCR amplification.

9. The board does not contest that a technique designated "nested PCR" is disclosed in several citations referred to on page 25 of the application, and thus was known to the skilled person at the day of filing. However, this does not result in a skilled person interpreting the amplification step disclosed in claim 1(g) as a PCR using nested primer sequences.

10. The only disclosure in the present application referring to nested oligonucleotide primers can be found on page 34, lines 16 to 25. This part of the description belongs to Example 3, disclosing the production of an anti-erythrocyte antibody. After a first 30 cycles **conventional** PCR amplification using downstream primers for a κ light chain constant region (SEQ ID. NO:1) and for the CH1 region of γ heavy chains (SEQ ID. NO:2), and an upstream primer for all κ variable regions (SEQ ID. NO:4)and a mixture of upstream primers (SEQ IDs. NO:5-12) *"able to recognize all heavy chain variable regions"* (page 33, lines 31 to 32), the amplified heavy chain DNAs are subjected to 21 cycles of a **further** PCR amplification, using the mixture of upstream primers as described above and a nested downstream primer *"specific for another portion of the CH1 region of all gamma chain constant regions (SEQ ID. NO:3).*

11. This disclosure in an example, referring to the use of specific primers defined by their respective nucleotide sequences, can also not corroborate appellants interpretation of the wording of claim 1(g), which the board considers to describe a "conventional" PCR amplification step as it is identically disclosed in document (5), page 937, left column.

12. Accordingly, the only remaining difference between the subject-matter of claim 1 and document (5) is that antibody-forming B cells or progeny thereof, which have been identified to produce a specific antibody, are used as starting material instead of selected hybridoma cells.

13. The appellants argued that a skilled person faced with the problem underlying the application and trying to bypass cell fusion technique and problems associated therewith, would not have applied the method of document (5) to B cells as he would not have had a reasonable expectation of success. Appellants pointed out that immortal hybridoma cells are more stable and therefore easier to handle than B cells, which latter were considered to contain uncertain levels of mRNA.

Appellants concluded that at the relevant date of the present application a strong prejudice existed in the art against applying the method of document (5) to non-hybridoma cells. This conclusion was supported by appellants technical expert at the oral proceedings before the opposition division.

14. The board, while taking into consideration appellants' line of argumentation and especially the submission of their technical expert, does, however, not agree.

Document (5) on page 936, left column, second full paragraph reads: *"The present work shows the generality of the mixed primer approach[21] and that total-RNA prepared from a single cell can be amplified by PCR to yield fragments that can be sequenced.* ***This level of sensitivity makes it possible to directly clone and sequence immunoglobulin genes from antigen-expanded B cell clones or from single plaque-forming cells."*** (emphasis added by the board). The paragraph finishes with the statement: *"This could prove to be a very powerful method for cloning antigen-specific human antibody genes that subsequently can be combined with the gene coding for the desired constant region, perhaps circumventing the need to generate human hybridomas."*

15. This, in the boards view can hardly be interpreted as a prejudice, but rather provides the skilled reader with motivation to apply the method to single B cells in order to solve the problem underlying the present invention, i.e. to produce antibodies not using hybridomas.

16. According to the established case law of the boards of appeal (cf eg T 386/94 OJ EPO, 1996, 658; T 923/92 OJ EPO; 1996, 564, T 333/97 5 October 2000), in cases where the prior art provides suggestions or incentives to do something and thus it may seem obvious for the skilled person to follow the indicated path, the question may arise whether the skilled person, based on scientific evaluation of the facts at hand would thereby have had a "reasonable expectation of success".

According to the finding in decision T 207/94 (OJ EPO, 1999, 273) an assumption or hypothesis about a possible obstacle to the successful realisation of a project must always be based on facts (point 34 of the Reasons for the Decision). Therefore, if something is not proven on a scientific basis to constitute an obstacle to carrying out an invention, it does not have an influence on the skilled person's expectation.

17. In the present case the board neither in document (5) nor in any other document on file can find a scientific basis for the existence of something that would have induced the skilled person not to follow the suggestion in document (5) and to apply the method to non-hybridoma-, and specifically, to B cells. Thus, when judged based on technical facts, a skilled person was not facing a prejudice or real obstacles, but had a reasonable expectation of success to produce antibodies with desired variable regions not using hybridoma cells.

18. On 5 December 1995, the appellants submitted an affidavit by Dr John W. Schrader, the designated inventor of the present application. Dr Schrader reported that the experimental methods of document (5) were replicated, but were directed to the amplification of immunoglobulin V_H cDNA's from a single primary IgM antibody-producing cell rather than from a single IgM antibody-producing hybridoma cell. On page 3, in point (4)(d) of the affidavit it is stated that the results of the experiment were negative, that the method of document (5) lacks the necessary sensitivity and is entirely unable to amplify immunoglobulin cDNA's from single primary human IgM antibody-forming cells.

19. The Board is of the opinion that the experiments carried out by Dr Schrader and commented in his affidavit fall within the scope of claim 1. This is confirmed in the affidavit (page 1, last paragraph), and is evident from a comparison of working steps (4)(a) to (4)(c) on pages 2 and 3 of the affidavit with points (a) to (i) of claim 1. Since these experiments gave negative results, the claim is open to a further objection under Article 56 EPC.

20. The ruling in decision T 939/92 (OJ EPO, 1996, 309) is applicable here. The competent Board held, that in view of the state of the art a technical effect solving a technical problem, which is the sole ground for an alleged inventive step had to be achieved by all embodiments falling within the scope of a claim (Reasons for the Decision Nos. 2.4 to 2.6). A claim covering embodiments not achieving said effect, and thus not solving the underlying problem, does not meet the requirements of Article 56 EPC, for everything falling within a valid claim has to be inventive.

21. In a further declaration by Dr John W. Schrader, filed by the appellants on 17 September 1999, it is stated that anti-influenza, and anti-tetanus toxin human monoclonal antibodies, as well as rabbit IgG antibodies *"specific for various antigens of diagnostic interest"*, all produced *"in experiments following the experimental methods of the above-identified application"*, showed unexpectedly high binding affinities, when compared with prior art antibodies.

In the light of this surprising result, the appellants argue in favour of an inventive step of the claimed method.

22. The Board cannot accept an inventive step on the basis of the evidence submitted with this declaration, as it does not contain experimental protocols allowing the Board to verify the relevance of the alleged results, all the more so as the present application does not report on the preparation of antibodies having any of the specific activities mentioned in the declaration.

23. In consequence, the subject-matter of claim 1 is not based on an inventive step, contrary to the requirements of Article 56 EPC.

First and Second Auxiliary Requests

24. Claim 1 of both requests refers in item (g) to the amplification of DNA using *"a nested primer"*.

As already mentioned in point (10) above, the only reference to a nested primer is found in example 3 (page 34, lines 16 to 25).

This passage of the description refers to specific oligonucleotide primers characterised by their nucleic acid sequences. The description as originally filed does not contain any statement from which a skilled reader can unambiguously deduce that the application generally refers to a method as claimed in claim 1 of both auxiliary requests, comprising an amplification step wherein use is made of nested primers.

25. The appellants on 17 September 1999 have filed four declarations signed by technical experts, and on 20 May 2003 a further declaration by one of these experts, all stating that in their understanding the disclosure of nested primers in the application as originally filed

is not restricted to the specific example, but is applicable to all circumstances of the invention.

26. The board, while not disregarding or discrediting the expert declarations, observes that they address the question whether or not a person skilled in the art would have regarded nested primers as useful tools in all circumstances of the invention. This question, which is answered by the four experts in the affirmative, is rather concerned with practical aspects of performing the claimed invention. The problem, if the application has been amended in a way that it contains subject-matter which extends beyond its content as originally filed, which is underlying Article 123(2) EPC, is a matter for the board to decide.

27. In their argumentation the appellants referred to the decision of the boards of appeal T 187/91 (OJ EPO, 1994, 572). This decison is concerned with a claim to a fiber optic amplifier, originally containing *"a plurality of pump light sources"*. During prosecution this feature has been changed to *"a pump light source"*. The board found that this amendment was in agreement with the requirements of Article 123(2) EPC, by pointing to a generic statement in a part of the description dealing with a preferred embodiment of the invention having three such light sources, which expressed that *"..while the drawings show three such light sources 60 mounted on the cone shaped rod 50, it will be understood that more or less sources 60 may be utilized."*

This situation cannot be compared with the present case, where in an example, referring to the

amplification of highly specific biological material, in an explicitly mentioned additional working step, the use of a specific primer, characterised by its nucleic acid sequence, is disclosed, and where the application as originally filed does not contain any statement referring to the general applicability of this specific teaching.

28. In decision T 1067/97 (4 October 2000) the board confirmed that if a claim was to be restricted to a preferred embodiment, it was not admissible under Article 123(2) EPC to extract isolated features from a set of features which had originally been disclosed in combination for that embodiment.

29. Thus in the light of facts of the present application and considering the relevant case law of the Boards of Appeal, claim 1 of both auxiliary requests contains subject-matter which extends beyond the content of the application as filed, contrary to the requirements of Article 123(2) EPC.

Reimbursement of appeal fees

30. According to Rule 67 EPC, it is a prerequisite for the reimbursement of appeal fees that the Board of Appeal considers an appeal to be allowable. As this prerequisite is not given in the present case, appellants request for reimbursement has to be rejected.

Order

For these reasons it is decided:

The appeal is dismissed.

The Registrar: The Chairwoman:

P. Cremona

U. M. Kinkeldey