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D E C I S I O N
of 3 February 2003

Case Number: T 1092/98 - 3.3.8

Application Number: 85303702.6

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IPC: C12N 15/64

Language of the proceedings: EN

Title of invention:

Methods for producing proteins and transformed cells, and DNA constructs for correcting host cell deficiencies and their preparation and use

Patentee:

ZymoGenetics, Inc.

Opponent:

Gist-brocades n.v.

Headword:

Transformed cells/ZYMOGENETICS

Relevant legal provisions:

EPC Art. 54, 56

Keyword:

"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:

-

Catchword:

-



Case Number: T 1092/98 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 3 February 2003

Appellant I: ZymoGenetics, Inc.
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Decision under appeal: Interlocutory decision of the Opposition Division
of the European Patent Office posted 23 September
1998 concerning maintenance of European patent
No. 0 171 142 in amended form.

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
M. B. Günzel

Summary of Facts and Submissions

I. The patent proprietors (appellants I) and the opponents (appellants II) lodged an appeal against the interlocutory decision of the opposition division dated 23 September 1998, whereby the European patent No. 0 171 142 was maintained on the basis of the second auxiliary request then on file, the main and the first auxiliary requests not being allowed, respectively, for lack of novelty and lack of inventive step.

II. Claims 1, 4, 11, 13 and 14 of the second auxiliary request read:

"1. A method for producing a foreign protein product in a eucaryotic host cell comprising the steps of:

a) transforming the host cell with a DNA molecule and

b) selecting the transformant from step a) by culturing the host cell on a selection growth medium

characterised in that

- in step a) the host cell has a deficiency in a gene which expression is essential for normal cell growth on a complex medium, and the DNA molecule comprises a gene as selectable marker which, when expressed, complements said deficiency, and a sequence coding for said foreign protein product, and

- in step b) the selection growth medium is a complex growth medium which need not contain antibiotics or heavy metals and need not be depleted of specific nutrients

and which method does not require a further selection on a special medium containing antibiotics or heavy metals or be depleted of specific nutrients."

"4. A method for producing transformed eucaryotic cells comprising the steps of:

a) subjecting host cells to transforming conditions in the presence of a DNA construct and

b) subjecting the cells from step a) to growth in a medium

characterised in that

- in step a) the host cells have a deficiency in a gene which expression is essential for normal cell growth on complex media, and the DNA construct comprises a gene as selectable marker which, when expressed, complements said deficiency, and

- in step b) the growth medium is a complex medium which need not contain antibiotics or heavy metals and need not be depleted of specific nutrients allowing transformed cells to remain viable and multiply while untransformed cells fail to multiply, due to said deficiency, and

which method does not require a further selection on a special medium containing antibiotics or heavy metals or be depleted of specific nutrients."

"11. A DNA construct for producing a foreign protein product in a eucaryotic host which comprises as a

selectable marker allowing selection on a complex medium a gene which, when expressed, complements a deficiency in a host cell, said deficiency being in a gene required for host cell division, cell wall biosynthesis, membrane biosynthesis, organelle biosynthesis, protein synthesis, carbon source utilisation, RNA transcription or DNA replication, and a DNA sequence coding for a foreign protein product, which is expressed in said host cell, which DNA sequence does not function as a selectable marker in said host cell, said protein product being selected from α -1-antitrypsin, interferons, insulin, proinsulin and tissue plasminogen activator."

"13. The DNA construct according to claim 11, characterised in that it comprises a plasmid pFPOT or a plasmid available from ATCC deposit numbers 20698, 20699, 20744 or 39685."

"14. A transformed strain characterised in that it contains a DNA construct selected from the constructs according to any of claims 11 to 13 or the construct pB5 (ATCC 20698), pFATPOT (ATCC 20699) or pMPOT2 (ATCC 20744) and expresses the foreign protein."

III. Both appellants filed a statement of grounds of appeal requesting that the decision of the opposition division be set aside, appellants I requesting the maintenance of the patent on the basis of the main or the first auxiliary request and appellants II requesting the revocation of the patent.

IV. On 19 November 2002, the board issued a communication pursuant to Article 11(2) of the rules of procedure of the boards of appeal with preliminary considerations on

the pending matters.

V. In reply thereto, appellants I filed new auxiliary requests II, IV and V and rearranged their requests.

VI. Oral proceedings took place on 3 February 2003. They were attended only by appellants I which filed a new main request (claims 1 to 16), appellants II having informed the board of their intention not to attend them.

The said new main request differed from the second auxiliary request which had been accepted by the opposition division (see section II, supra) only in that (i) in claim 13 references to plasmids other than the one available from ATCC deposit number 20699 and (ii) in claim 14 all the references to specific constructs were deleted.

VII. Appellants I submitted that the methods of claims 1 and 4 of the request at issue were new as they were not disclosed in either of documents (1) and (3) (see section IX, infra), a difference being that in these documents the reported experiments relied on the use of a selection system involving not a complex but a well-defined and, therefore, expensive medium. Furthermore, in the situation of document (3) where a complete medium was used, cell growth was observed at the restrictive temperature of 38°C, ie a temperature which did not allow normal growth of the host cells.

Taking document (1) as the closest prior art, appellants I defined the technical problem solved by the invention as the provision of an efficient expression system which contrary to the selection

system of document (1) did not require selection of transformants on an expensive medium, the said problem being solved in a non-obvious manner by the methods claimed.

VIII. In their statement of grounds of appeal, appellants II considered that the subject-matter of claims 1 and 4 of the second auxiliary request of the decision under appeal was not new and did not involve an inventive step having regard to documents (1) and (3) (see section IX, *infra*). Other independent claims were not referred to.

Claims 1 and 4 lacked novelty over document (3) because the selection of transformants was independent of the selection growth medium composition, as it required only a temperature change. Selection growth medium was not an essential feature of document (3). The medium used in the said document was nevertheless equivalent to the "complex" media of the claims. In fact, the selection growth medium was a poorly-defined medium. Indeed, it was one of the media of document (A) (see section IX, *infra*). The distinction between "complex" and "complete" medium upon which the opposition division relied was not rigorous. Important was that the medium of document (3) was a poorly-defined medium, this being within the scope of the claims which contained no limitation to a medium without any defined components or supplements.

Document (1) was also relevant for novelty because it disclosed a selection system similar to that of document (3), a medium requirement being not an essential element.

As for inventive step, appellants II argued that a person skilled in the art would have regarded it as obvious to substitute a complete medium of the invention for the complete medium of document (3).

IX. The following documents are referred to in the present decision:

(1): G. Kawasaki and D. G. Fraenkel, *Biochem. Biophys. Res. Comm.*, Vol. 108, No. 3, 15 October 1982, pages 1107 to 1112;

(3): K. A. Nasmyth and S. I. Reed, *Proc. Natl. Acad. Sci. USA*, Vol. 77, No. 4, April 1980, pages 2119 to 2123;

(A): L. H. Hartwell, *J. Bacteriol.*, Vol. 93, No. 5, May 1967, pages 1662-1670;

Document (A) is citation (11) of document (3). It was referred to by appellants II in their statement of grounds of appeal.

X. Appellants I requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request filed at the oral proceedings.

Appellants II requested in writing that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Formal requirements

1. In its decision the opposition division has considered that the requirements of Articles 84, 123(2) and 123(3) EPC were met by the second auxiliary request then on file. This finding was not contested by appellants II.
2. The request at issue differs from the said request only in respect of claims 13 and 14 wherein the amendments have resulted in the deletion of (i) in claim 13, references to DNA constructs not containing a DNA sequence encoding a foreign protein product, and (ii) in claim 14, all the references to the DNA constructs.
3. Said amendments were necessary in order to remove an inconsistency with claim 11 which is back-referred in each of claims 13 and 14 as well as a redundancy between claims 13 and 14 (there was no need to repeat in claim 14 which is dependent on claim 13 DNA constructs already mentioned in claim 13). Said amendments have not introduced subject-matter which was not already present in the application as filed and have resulted in a limitation of the protection conferred.
4. Therefore, the board is satisfied that, account being taken of the amendments the main request contains compared to the claims as granted, the main request as a whole meets the requirements of Articles 84, 123(2) and 123(3) EPC.

Novelty of claims 1 and 4 vis-à-vis document (1)

5. Document (1) reports on experiments aiming at cloning yeast glycolysis genes by complementation. Cells of various leu2 and glycolysis mutant strains of *Saccharomyces cerevisiae* are transformed with a yeast

DNA pool in YEp13, a high copy plasmid carrying the selectable LEU2 wild type gene.

Using **a synthetic medium** containing yeast nitrogen base, glucose, adenine, uracil and eleven amino acids, but no leucine, transformants with a glycolysis wild phenotype are obtained by complementation, with simultaneous selection for (i) growth on glucose and (ii) leucine prototrophy.

6. Each of claims 1 and 4 is **a claim to an activity**, respectively the activity of producing a foreign protein and the activity of producing transformed cells. Neither of said activities is the gist of the experiments reported in document (1), which, indeed, is concerned with the cloning of yeast genes by complementation, the purpose of the authors not being to devise a method for producing a protein or producing transformed cells.
7. In addition, the claims at issue refer as an essential feature of the claimed method to the use of a **complex** medium which is defined in the specification (see lines 24 to 26 on page 3) as a medium "*in which the nutrients are derived from products whose composition is not well defined, such as crude cell extracts, meat extracts, fruit juice, serum, protein hydrolysates, etc.*" whereas a synthetic, ie well-defined, medium is employed in document (1) (see page 1108).
8. The stated differences are sufficient to lead to the conclusion that the methods of claims 1 and 4 are not disclosed in document (1).

Novelty of claims 1 and 4 vis-à-vis document (3)

9. Document (3) reports on experiments aiming at cloning a yeast cell-cycle gene. Cells of a trp1 and CDC mutant strain of *Saccharomyces cerevisiae* were transformed with two plasmids referred to as YRp7-CDC28(2) and YRp7-CDC28(3). The CDC mutation of the strain, cdc28^{ts}, is a conditionally lethal mutation which leads to stage-specific arrests of the cell division cycle.

Both plasmids carry the TRP1 wild type gene, which encodes a functional anthranilate isomerase, and the CDC28 wild type gene.

According to a first embodiment, transformants are selected on a **tryptophanless** medium at the permissive temperature of 23°C (see last full paragraph of page 2120 and Table 1 on page 2121), thereby selecting for tryptophan prototrophy TRP⁺ only.

According to a second embodiment, transformants are selected on a **complete** medium at the restrictive temperature of 38°C (see last full paragraph of page 2120 and Table 1 on page 2121), thereby selecting for CDC⁺ phenotype.

10. As in document (1) the gist of document (3) is the cloning of yeast genes by complementation, said document being not concerned with the activities to which claims 1 and 4 relate.
11. Whereas it is unquestionable that a tryptophanless medium is not a complex medium in the sense of the invention, the question at issue which remains to be answered is whether, as alleged by appellants II, the complete medium of document (3) may be regarded as a

complex medium of the invention.

As the composition of the complete medium is not at all described in document (3), the medium being only referred to once in Table 1 (see page 2121), its true composition can only be speculated.

In this respect, appellants II have only speculated but not proven that the complete medium is one of the three media described in document (A) (see page 1663), ie a medium prepared by supplementing products whose composition is not well defined (a yeast extract, a peptone and a yeast nitrogen base were used), with some specific nutrients. Their allegation relies on the admission that the sentence on page 2119 of document (3) (see the section entitled "Organisms, DNAs, Enzymes and Media") that reads: **"All media used for the culture of yeast cells have been described in document (11)"** also encompasses all the media used for the **selection** of transformed yeast cells, which in fact is not the case because the tryptophanless medium for obvious technical reasons could not be one of the media of document (A). Another speculative reasoning could as well lead to the conclusion that, as in Table 1 results of **comparative** experiments are reported in which only the effect of tryptophan (presence or absence) has been assessed, the complete medium, with a view of avoiding comparisons being biased, has been directly derived from the tryptophanless medium by supplementing it with tryptophan and, therefore, is a well-defined medium.

At any rate, as the composition of the complete medium of document (3) cannot be established with certainty, it cannot be concluded that said medium is a complex medium in the sense of the invention.

12. Therefore, as the claimed methods are not derivable from document (3) and also in view of the fact that the medium of the document is not a complex medium according to the invention, the board concludes that the methods of claims 1 and 4 are not disclosed also in document (3).

13. Thus, the subject-matter of claims 1 and 4 is new.

Inventive step of claims 1 and 4

14. As afore-mentioned (see points 6 and 10, supra), documents (1) and (3) are not concerned with the activities of producing a protein and of producing transformed cells. They do not deal with the technical problem faced by the invention which can be regarded as the provision of a selection system which ensures that DNA constructs are maintained within a culture of transformed cells in alternative to the usual systems based either on antibiotic resistance or nutritional requirements (cf description of patent specification, page 2, lines 19 to 29). As such, neither of them is really qualified to represent the closest prior art for the methods claimed. As a matter of fact, the background art cited in the patent specification (cf loc. cit.) constitutes a more appropriate starting point for the evaluation of inventive step.

15. Having regard to said prior art knowledge on how to select transformants for further culture or production of a foreign protein, in the board's judgment a person skilled in the art would have found no incentive in the art to (i) develop a selection system which is based on complementation of a deficiency in a gene the expression of which is essential for normal cell growth

upon growth on a complex medium, and (ii), thereby, arrive at the inventions of claims 1 and 4.

16. Therefore, the board comes to the conclusion that the subject-matter of claims 1 and 4 involves an inventive step.

Novelty and inventive step of claim 11

17. Claim 11 is directed to a DNA construct which comprises two genes, one being a gene which, when expressed, complements a deficiency in a host cell, and the other being a gene coding for one of the proteins consisting of α -antitrypsin, interferons, insulin, proinsulin and tissue plasminogen activator.

18. Such a construct is not disclosed in either of documents (1) and (3) (see points 5 and 9, supra).

19. In the board's judgement, a person skilled in the art would have found no incentive either in any of documents (1) and (3) or in their combination to prepare such a construct which is a key-tool for performing activities with which said documents are in any case not concerned. This is also in agreement with the finding of the opposition division on the identical claim of the second auxiliary request then accepted, a finding which appellants II did not dispute in their statement of grounds of appeal.

20. Thus, the subject-matter of claim 11 of the main request at issue is new and involves an inventive step.

Conclusion

21. For the above reasons, the main request as a whole complies with the requirements of Articles 54 and 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted to the opposition division with the order to maintain the patent with the claims of the main request filed during the oral proceedings, description pages 3 and 6 to 16 as granted, description pages 2, 4 and 5 as received on 19 February 1998 and drawings as granted.

The Registrar:

The Chairman:

A. Wolinski

L. Galligani