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D E C I S I O N
of 8 May 2003

Case Number: T 0620/99 - 3.3.4

Application Number: 91900640.3

Publication Number: 0452484

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Language of the proceedings: EN

Title of invention:
Production of proteins using homologous recombination

Patentee:
CELL GENESYS, INC.

Opponents:
APPLIED RESEARCH SYSTEMS ARS HOLDING NV
Institut Pasteur
Roche Diagnostics GmbH

Headword:
Homologous recombination/CELL GENESIS INC.

Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 123(2), 123(3)

Keyword:
"Main request, inventive step (no)"
"Auxiliary request 1, novelty, inventive step, sufficiency (yes)"

Decisions cited:
G 0007/95, T 0014/83, T 0248/85, T 0409/91, T 0270/94

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0620/99 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 8 May 2003

Appellant: (Proprietor of the patent)
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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 12 April 1999 revoking European patent No. 0452484 pursuant to Article 102(1) EPC.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: M. R. J. Wieser
S. C. Perryman

Summary of Facts and Submissions

I. The appeal was lodged by the patent proprietors (appellants) against the decision of the opposition division, whereby European Patent No. 0 452 484 was revoked under Article 102(1) EPC. It had been opposed by three parties under Article 100(a) EPC on the ground of lack of inventive step (Article 56 EPC) and Article 100(b) EPC for lack of sufficiency of disclosure (Article 83 EPC).

II. Claims 1 and 7 of the patent as granted read:

"1. A method for producing mammalian proteins comprising:

growing mammalian secondary expression host cells comprising multiple copies of an amplifiable region comprising a target gene heterologous to said secondary expression host and expressing a protein of interest and an amplifiable gene, whereby said target gene is expressed and said protein is produced;
wherein said secondary host expression cells are produced by the method comprising:
transforming primary mammalian cells comprising said target gene with a construct comprising an amplifiable gene and at least one flanking region of a total of at least about 150 bp homologous with a DNA sequence at the locus of the coding region of said target gene to provide amplification of said target gene, wherein said amplifiable gene is at a site which does not interfere with the expression of said target gene, whereby said construct becomes homologously integrated into the

genome of said primary cells to define an amplifiable region;
selecting for primary cells comprising said construct by means of said amplifiable gene or other marker present in said construct;
isolating DNA portions of said genome from said primary cells, wherein said portions are large enough to include all of said amplifiable region;
transforming secondary expression host cells with said primary cell DNA portions and cloning said transformed secondary expression host cells to produce clones of said secondary expression host cells differing in said DNA portions present in said secondary expression host cells;
selecting clones of said mammalian secondary expression host cells comprising said amplifiable region; and
amplifying said amplifiable region by means of an amplifying agent, wherein said amplifying is prior to said isolating or after said selecting and prior to said growing.

7. A method for producing cells for expression of a heterologous protein in culture, said method comprises:

transforming mammalian primary cells comprising said target gene with a construct comprising an amplifiable gene and at least one flanking region of at least about 150 bp homologous with a DNA sequence within 10 kb of the coding region of said target gene, wherein said amplifiable gene is at a site which does not interfere with the expression of said target gene, whereby said construct becomes homologously integrated into the genome of said primary cells to define an amplifiable

region comprising said amplifiable gene and said target gene in said genome;
selecting for primary cells comprising said construct by means of said amplifiable gene or other marker present in said construct;
isolating DNA portions of said genome from said primary cells, wherein said portions are large enough to include all of said amplifiable region;
transforming mammalian secondary expression host cells with said primary cell DNA portions, wherein said secondary expression host cells are of a different species from said primary host cells, and cloning said transformed secondary expression host cells to produce clones of said secondary expression host cells differing in said DNA portions present in said secondary expression host cells;
selecting clones of said mammalian secondary expression host cells comprising said amplifiable region; and
amplifying said amplifiable region by means of an amplifying agent, wherein said amplifying is either prior to said isolating or after said selecting."

III. The opposition division decided that, while the requirements of sufficiency of disclosure were met, the subject matter of the claims as granted did not involve an inventive step in the light of the following documents:

(17) Kaufman, R.J., "High level production of proteins in mammalian cells"; in "Genetic Engineering; Principles and methods", vol.9, Plenum Press, New York (US), 1987, pages 155-186

(24) Trends in Genetics, Vol. 5, No. 3, 1989, pages 70-76

IV. Oral proceedings took place on 8 May 2003. At the oral proceedings respondents I (opponents 01) introduced a new ground of opposition, lack of novelty of claim 1 as granted. The appellants filed auxiliary request 1, which differed from the claims as granted in claim 1 only.

Claim 1 of auxiliary request 1 reads:

"A method for producing mammalian proteins comprising:

producing mammalian secondary expression host cells comprising multiple copies of an amplifiable region comprising a target gene heterologous to said secondary expression host and expressing a protein of interest and an amplifiable gene by the method comprising:
transforming primary mammalian cells comprising said target gene with a construct comprising an amplifiable gene and at least one flanking region of a total of at least about 150 bp homologous with a DNA sequence at the locus of the coding region of said target gene to provide amplification of said target gene, wherein said amplifiable gene is at a site which does not interfere with the expression of said target gene, whereby said construct becomes homologously integrated into the genome of said primary cells to define an amplifiable region;
selecting for primary cells comprising said construct by means of said amplifiable gene or other marker present in said construct;

isolating DNA portions of said genome from said primary cells, wherein said portions are large enough to include all of said amplifiable region;
transforming secondary expression host cells with said primary cell DNA portions and cloning said transformed secondary expression host cells to produce clones of said secondary expression host cells differing in said DNA portions present in said secondary expression host cells;
selecting clones of said mammalian secondary expression host cells comprising said amplifiable region;
growing said mammalian secondary expression host cells whereby said target gene is expressed and said protein is produced; and
amplifying said amplifiable region by means of an amplifying agent, wherein said amplifying is prior to said isolating or after said selecting and prior to said growing."

V. Besides the documents mentioned in section (III) above the following documents are referred to in this decision:

(3) Ann.Rev.Biochem., Vol. 50, 1981, pages 533-554

(9) Biotechnology, Vol. 3, 1985, pages 561-566

(10) Mol.Cell.Biol., Vol. 5, 1985, 1750-1759

(11) Proc.Natl.Acad.Sci.USA, Vol. 83, 1986, p.3136-3140

(16) Cell, Vol. 51, 1987, pages 503-512

(18) Proc.Natl.Acad.Sci.USA, Vol. 85, 1988, p.8583-8587

(20) Nature, Vol. 336, 1988, pages 348-352

VI. The submissions by the appellants may be summarised as follows:

They did not agree to the introduction of lack of novelty as a new ground for opposition.

The objections under Article 83 EPC were without substance, the invention was disclosed in a sufficient manner, as already correctly decided by the opposition division. Moreover, Respondents I should not be allowed to argue under Article 83 EPC, as this ground of opposition was not raised by them.

Although it was accepted that document (17) might be the best starting point for assessing inventive step, it was stated that in the present case, as in all cases dealing with pioneer inventions, it was difficult to define the closest prior art. The decision of the opposition division was based on a misinterpretation of the claims, which were directed towards targeting genes already present in the primary cell. There was no basis for combining documents (17) and (24), because the latter was concerned with unrelated subject-matter. Homologous recombination, as in the patent in suit, was not regarded as being an equivalent to co-transfection, as disclosed in document (24).

VII. The submissions made by the Respondents may be summarised as follows:

VIII. Respondents I stated, that the skilled person, when trying to perform the invention, was faced with undue burden, as the patent did not disclose how cells, not growing readily in culture, could be used in a process comprising a homologous recombination step, which, as disclosed in document (18), was a very rare event, just detectable even when using cells that grew very well in culture.

Claim 1 of the main request, formally drafted as method-claim, was in fact a mixture of a method- and a product-by-process-claim, and as such not novel over document (17). The relevant case law of the Boards of Appeal establishing the criteria for assessing novelty of claims defining a product by a process for its production had to be applied in the same way to a mixed category claim.

The wording of the claims could not be interpreted as meaning that the target gene must be endogenous to the primary cell. Starting from document (17) as closest state of the art, the skilled person would have consulted document (24) referring to a technically neighbouring field, and would have arrived at the claimed subject-matter by considering homologous recombination as an obvious alternative to co-transformation. If the board came to the conclusion that the claims were restricted to target genes endogenous to the primary cell, document (24) was considered to be the most promising starting point for an objection under Article 56 EPC

Claim 1 of auxiliary request 1 was not clear.

IX. Respondents II (opponents 02) argued that the independent claims, stating that homologous recombination took place at the coding region of the target gene, which had to result in disturbance of this region, did not contain working steps to compensate for this disturbance, and thus missed an essential feature. In analogy to the decision T 409/91, holding that all features necessary for solving a technical problem were essential features and must be in the claims, the invention was insufficiently disclosed.

The claims were not limited to target genes endogenous to the primary cell. Document (17), the closest state of the art, by referring on page 174 to document (10), showed the functional equivalence of co-transfection and homologous recombination in the context of the contested patent. The transfer of DNA to the secondary expression host did not add anything to an alleged inventive concept.

In the light of document (24), disclosing targeting and selecting of genes of interest without using cDNA, the claimed subject-matter was obvious, even when assuming that it was restricted to endogenous genes.

Claims 12 and 13 referring to the use of DHFR negative cells, which according to document (11) are of very limited use only, did not solve the technical problem. Moreover, the claimed method did not solve the problems caused by the work and time intensive cloning and sequencing of target genes, since, as the examples showed, only very well characterized genes were expressed.

Claim 1 of auxiliary request 1 did not meet the requirements of Article 123(2) EPC.

X. Respondent III (opponent 03) considered the embodiment of the claimed method, according to which amplification took place in the primary cell, whereafter multiple copies of the amplifiable region were transferred to the secondary cell, as not sufficiently disclosed, and by reference to document (3) as not workable.

Either document (17) or (10) or (9) was the closest state of the art. Upon combination with document (24) it was obvious to arrive at the claimed subject-matter, which could not be interpreted as being restricted to target genes being endogenous to the primary cell.

Claim 1 of auxiliary request 1 was objected under Article 123(2) EPC.

XI. The appellants (patentees) requested that the decision under appeal be set aside and that the patent be maintained as main request as granted or as auxiliary request on the basis of the set of claims filed as auxiliary request 1 or auxiliary request 2 at the oral proceedings on 8 May 2003.

The respondents (opponents) requested that the appeal be dismissed.

Reasons for the Decision

Main request

1. *Sufficiency of disclosure - Article 83 EPC*

Lack of sufficiency of disclosure was raised as ground of opposition within the time limit given in Article 99(1) EPC by respondents II and III, and was found to be sufficiently substantiated for the purpose of Rule 55(c) EPC by the Opposition Division.

According to Article 99(4) EPC "Opponents shall be parties to the opposition proceedings as well as the proprietor of the patent". Thus several admissible oppositions do not initiate a corresponding number of parallel opposition proceedings but only a single one. Each opponent, in the present case in particular respondent I, can rely on an opposition ground duly submitted by other opponents and communicated to all the parties in accordance with Rule 57(2) EPC, both in the opposition proceedings and in any subsequent appeal proceedings (see decision T 270/94, 22 January 1998).

2. The patent in suit states in column 3, lines 6 to 7, that "the primary cell may be any mammalian cell of interest, particularly mammalian cells which do not grow readily in culture".

Document (18) relates to targeted gene mutation by site-directed homologous recombination (abstract). The results (page 8584, right column; page 8536, left column, Table 1) show, that homologous recombination is a very rare event.

Document (18) is concerned with targeted mutation of genes in mouse embryonic stem cells, and it does not mention amplifiable genes at all. Although it states that high cell numbers have to be treated in order to detect the rare event of homologous recombination, it does not state that mammalian cells not readily growing in culture are unsuitable for this purpose. It provides no factual basis for assuming that a skilled worker when carrying out the invention as claimed will be faced with an undue burden.

3. Claims 1 and 7 refer to the transformation of primary mammalian cells with a construct comprising an amplifiable gene and at least one flanking region of at least about 150 bp homologous with a DNA sequence at the locus of the coding region of said target gene. Moreover it is said that said amplifiable gene is at a site which does not interfere with the expression of said target gene.

Respondents II noted that while homologous recombination according to the wording of the independent claims can take place within the coding region of the target gene, which inevitably leads to disturbance of said region, the claims do not require that the targeting construct contains elements to compensate for any such disturbance and to reconstitute a functional target gene. By referring to the statement in decision T 409/91 (OJ EPO 1994,653), that the protection conferred by a patent should correspond to the technical contribution to the art made by the disclosure, the independent claims were considered to

lack an essential feature of the invention, which accordingly was not sufficiently disclosed.

The board does not agree. Decision T 409/91 itself makes clear that for sufficiency it is the whole description and not just the wording of the claims that needs to be considered. Further it is the consistent case law of the boards of appeal since decision 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, and not the claims alone.

The patent in suit in its two examples teaches the introduction of compensatory elements as compensation for elements lost during homologous recombination at the locus of the coding region. While the DNA vector of example 1 contains an additional t-PA promoter sequence (page 5, column 8, lines 31 to 33), the construct of example 2 contains a CMV promoter (figure 3), which is placed before the targeted gene's coding sequence and which, once the whole construct is inserted via homologous recombination into the primary cell, takes the place of the 'lost' promoter.

In the case of the present invention, claim 1 refers to a process for expressing a protein encoded by a target gene, including the step of inserting into a host a construct via homologous recombination at the locus of the coding region of a target gene. This insertion can take place at various regions, at the 3' or the 5' adjacent regions of the coding region or also directly at the coding region. In this latter case the application teaches in the examples which steps the

skilled reader should take in order to compensate for elements lost as a result of homologous recombination to reconstitute a functional target gene.

Thus, the board considers that the skilled person is given sufficient information to carry out the invention claimed.

4. The independent claims contain two alternative embodiments concerning the time when amplification of the amplifying region takes place. This event may either occur in the primary cell or in the secondary cell. If amplification happens in the primary cell only, this means that in the following step the secondary expression host cells have to be transformed with multiple copies of the amplified region.

This latter embodiment is not exemplified in the patent and, according to respondents III, referring to document (3), it is not feasible.

Document (3) is a review article published 1981, eight years before the claimed priority date and refers to chromosomal mediated gene transfer for transferring genetic material from a donor somatic cell to a recipient. In the passage bridging pages 536 and 537 the document discusses the size of the transferred genetic material. It is stated that most transgenomes are of subchromosomal nature, and that only small regions of DNA could be transferred while flanking regions were often lost. The document reports problems regarding stable expression of the transferred phenotype and of high rate loss of multiple copies of

the transgenome (page 545, first line and page 551, second paragraph).

The cited passages of document (3) might be considered as expressing doubts and uncertainties with regard to the possible size of transgenomes and their stability in hosts only. In order to convince the board that these considerations are serious indications for a lack of sufficiency of disclosure they had to be based on verifiable facts, such as experiments. None such have been filed.

Moreover, the board notes that the results discussed in document (3) were not obtained under experimental conditions comparable to those applied in the patent. While in both examples of the patent a selective pressure is provided on the transformed secondary recipients (page 6, column 9, lines 16 to 18 and page 7, column 12, lines 12 to 17), no such selective pressure is described in document (3). Consequently, the board cannot accept document (3) as evidence that the transfer of multiple copies of an amplified region cannot be performed.

5. The board concludes that the invention according to the claims of the main request is disclosed in a manner sufficiently clear and complete for it to be carried out by a skilled person according to the requirements of Article 100(b)EPC.

6. *Admissibility of a new ground of opposition - Article 54 EPC*

Lack of novelty has not been raised as ground of opposition by the respondents within the nine month opposition period, but has first been mentioned by respondent I at the oral proceedings. It was argued that claim 1 was not novel over document (17), which has been cited by respondents I in their notice of opposition and was considered as closest state of the art in the proceedings before the opposition division.

The appellants did not agree to the introduction of this new ground for opposition.

In the decision G 7/95 (OJ EPO 1996, 626) the Enlarged Board of Appeal decided, that in case where a patent has been opposed under Article 100(a) EPC on the ground that the claims lack an inventive step in view of documents cited in the notice of opposition, the ground of lack of novelty based upon Articles 52(1) and 54 EPC is a fresh ground for opposition and accordingly may not be introduced into the appeal proceedings without the agreement of the patentee. However, the allegation that the claims lack novelty in view of a closest prior art document may be considered in the context of deciding upon the ground of lack of inventive step, which the board will do.

7. *Inventive step - Article 56 EPC*

Claim 1, referring to a method, comprises an introductory statement followed by two separate parts. The first part, starting with "growing mammalian

secondary cells" and ending with "whereby said target gene is expressed and said protein is produced" has the form of a conventional process claim. It is undisputed between the parties that this first part of claim 1 indicates the subject-matter of the invention and those technical features which are necessary for the definition of the claimed subject-matter, but which are part of the state of the art, in the present case of document (17). This is in accordance with the requirements of Rule 29(1)(a) EPC.

8. Claim 1 is directed to "A method for producing mammalian proteins comprising: growing mammalian secondary expression host cells comprising multiple copies of an amplifiable region comprising a target gene heterologous to said secondary expression host and expressing a protein of interest and an amplifiable gene, whereby said target gene is expressed and said protein is produced; wherein said secondary host cells are produced by the method comprising [six consecutive working steps as set out]".

Mammalian secondary expression host cells having these characteristics are described in document (17), where a heterologous target gene and an amplifiable gene are incorporated into a host cell by co-transformation. The transfected DNA becomes ligated together inside the cell and subsequently integrates into host chromosomal DNA as a unit which can be co-amplified (page 160, lines 40 to 43).

9. The mammalian secondary expression host cells in document (17) are not, as a product, different from those referred to in present claim 1. The six step

production process of the mammalian secondary expression host cells is not a required feature of the method of claim 1 as presently phrased, but is merely used to define the product features of the mammalian secondary expression host cells. However it is the established case law of the Boards of Appeal that a product is not new merely because it has been made by a new process (cf Decision T 248/85, OJ EPO 1986, 261). The mammalian secondary expression host cells in document (17) are also to be grown to produce the protein of interest. On page 174, third paragraph document (17) states that these cells express high levels of various proteins from heterologous genes. This statement corresponds exactly to the process as disclosed in the first part of present claim 1 (see point (7) above). Thus claim 1 is directed to a known use of a known product, and so it lacks novelty, and thus inventive step.

10. The board comes to the conclusion that the subject-matter of claim 1 does not meet the requirements of the EPC.

Auxiliary request 1

11. *Formal requirements - Articles 84, 123(2), 123(3) EPC*

Respondent's I opinion, that claim 1 is unclear since it misses a logical order, is not shared by the board. The claim refers to a method consisting of various working steps which are listed chronologically, with the only exception of the amplifying step which is placed at the end of the claim. Since this particular working step comprises two alternative embodiments,

i.e. it may be carried out at two different times of the method, its positioning at the end of the claim is justifiable to avoid unnecessary repetitions.

12. The board can also not agree with respondents II, who argued that claim 1 has been amended in such a way that it contains subject-matter which extends beyond the content of the application as filed. The objected amendments were the change of the term "growing" into "producing" at the beginning of the claim, and the transfer of the passage referring to the expression of the target gene and the production of the protein, from the first paragraph to the end of the claim. No infringement of Article 123(2) can be seen to result from this regrouping of the otherwise unchanged working steps, in particular the board does not agree that claim 1 now refers to primary cells not containing the target gene.

13. The fact that the working step referring to the growing of the secondary expression host cells, no longer explicitly contains the feature that the cells contain multiple copies of an amplifiable gene, is not considered to extend the protection conferred by the claims as granted (as argued by respondents III). The growing step refers to "said mammalian expression host cells", which are disclosed at the beginning of the claim as comprising said multiple copies.

14. In consequence, the claims of auxiliary request 1 meet the requirements of Articles 84, 123(2) and 123(3) EPC.

15. *Sufficiency of disclosure - Article 83 EPC*

The conclusions drawn by the board with regard to sufficiency of disclosure (Article 83 EPC) of the main request, apply to auxiliary request 1 (see point 5 above).

16. *Novelty - Article 54 EPC*

Claim 1 relates to a method characterised by a number of different working steps. Its subject-matter is not disclosed in the cited prior art documents and is therefore novel under Article 54 EPC.

17. *Inventive step - Article 56 EPC*

Much emphasis was put by the parties on the interpretation of the wording of independent claims 1 and 7. It was controversly discussed whether the term "primary mammalian cells comprising said target gene" had to be interpreted in the sense that said target gene was endogenous to the primary cell, i.e. that it was naturally present therein, or, if it also comprised target genes which had been introduced into the primary cell by way of recombinant DNA technology.

The appellants argued that it was not in line with the specification to interpret the claims in a sense that they encompass the expression of target genes not naturally present in the primary cell. The term "primary cell" had to be seen in the sense of meaning a "naive cell". It was clear from the description that it was the aim of the invention to manipulate an endogenous gene by forming an in situ expression

cassette without the need of fully characterizing, sequencing and cloning a desired gene to finally get a cDNA which then may be transformed into an expression host cell. To follow this latter way of procedure was considered to be illogical, as transforming a primary cell with the cDNA construct obtained by conventional recombinant DNA technology, followed by a second transformation step wherein said DNA is transformed into a secondary host cell for its expression did not make sense. A skilled person would see no reason for such first transformation step but rather would directly go to the expression host.

18. This interpretation was not accepted by the respondents, who considered the term "primary cell" to have the meaning as being a "first cell" in a series of steps. The specification was no basis allowing a skilled reader to interpret the claims as being restricted to target genes endogenous to such primary cells. The possible intention of the appellants was not relevant when constructing the scope of the claims.

19. The board does not consider this point to be decisive for its decision on inventive step of the claimed subject-matter as presented below, so that no decision in this respect is considered to be necessary.

20. It is undisputed between the parties that document (17) represents the closest state of the art. The board agrees.

Document (17) is a review article published two years before the priority date of the patent in suit, having the title "High level production of proteins in mammalian cells" and is considered to be a technically realistic starting point for assessment of inventive step. The document discusses in its starting passage general problems of introduction of genetic material into cells, describes viral transduction systems and DNA-mediated gene transfer (pages 155-161). Starting on page 161, selection of stable transformants using drug resistance markers is dealt with. On pages 165 to 166 the Dyhrofolate Reductase (DHFR) selection system is described. The primary advantage of this system is said to be its ability to select for cells which contain an amplified copy number of DHFR genes due to its amplification under appropriate drug selection, i.e. increasing amounts of methotrexate (page 165, last par.).

Page 166, first full sentence reads: "When DHFR is used as a cotransformation selection marker in DHFR-deficient CHO cells, it is possible to coamplify and co-express the heterologous gene by selection of the transformants in increasing concentration of methotrexate". A few lines down on the same page it is said, that "Although most frequently used as a recessive marker in the DHFR-deficient CHO cells, there have been several adaptations which have made DHFR a useful dominant selectable and amplifiable genetic marker."

A chapter headed "Optimizing expression of heterologous genes" starts on page 168. Gene amplification is dealt with in detail on pages 170 to 174. It is stated that

gene amplification appears to be ubiquitous in nature and that many, if not all genes become amplified at some frequency. In Table 1 on page 171, a list of amplification systems in drug resistant mammalian cells, including DHFR-methotrexate, is given. This chapter ends on page 174 with the passage reading: "Coamplification of heterologous genes with DHFR has been successfully used to obtain a number of cell lines that express high levels of a protein from heterologous genes. Some examples include human tissue plasminogen activator (158), human gamma interferon (166,167), human beta-interferon (191), human factor IX (168), the herpes simplex glycoprotein D (169), the alpha and beta subunits of bovine luteinizing hormone (170)."

Reference (158) cited in document (17) corresponds to document (10) in the present proceedings. It is a document published by the authors of document (17) and refers to "Coamplification and Coexpression of Human Tissue-Type Plasminogen Activator and Murine Dihydrofolate Reductase Sequences in Chinese Hamster Ovary Cells". Two expression vectors, one containing human t-PA cDNA, the other DHFR cDNA were co-transfected into CHO DHFR-deficient cells and high level expression of human t-PA is achieved in the transfected cells. According to page 1750, left column, first sentence "The gene sequences cotransfected with the DHFR gene are frequently integrated adjacent to the DHFR gene, and thus subsequent amplification of the DHFR gene results in coamplification of the adjacent DNA".

To summarize, document (17) discloses a first aspect of the claimed method, namely coamplification and coexpression of an amplifiable gene and a heterologous target gene in a selected host cell.

A similar approach is described in document (9), referring to expression of amplified hepatitis B virus surface antigen genes in CHO cells, wherein the DHFR cDNA and the HBV sequences were on the same plasmid (abstract and figure 1).

21. The problem underlying the patent in suit is described on page 2, column 1, lines 39 to 43 as "developing alternative techniques for producing proteins of interest in culture with cells which provide for economic and efficient production of the desired protein and, when possible, appropriate processing of the protein product."

22. The formulation of the problem that the board regards as appropriate is developing alternative techniques for producing proteins of interest in culture with cells. The board does not regard a reference to economic and efficient production as necessary or appropriate because the evaluation of what is economic or efficient is subjective in the absence of precise criteria of how this is to be assessed.

This problem of developing alternative techniques for producing proteins of interest in culture with cells is solved by providing a method according to claim 1. This method is distinguished from the process of the closest prior art by transforming a construct comprising an amplifiable gene into a primary mammalian cell

comprising a target gene by homologous recombination, followed by transformation of the complete expression cassette formed in situ in the primary cells into secondary expression host cells.

23. The opposition division stated, that homologous recombination was an alternative method to bring the amplifiable gene in contact with the gene of interest, having the same technical effect as transfecting a host with an amplifiable gene and a target gene, positioned either on the same plasmid (9), or on two different plasmids (10). Thus these methods were found to be functional equivalents. Consequently a skilled person would turn to document (24), where he would get information prompting him to apply the technique of homologous recombination in the process described in document (17), and would arrive at the claimed subject-matter in an obvious way.

24. Document (24), a review article published six months before the priority date, having the title "The New Mouse Genetics, Altering the Genome by Gene Targeting", refers to gene targeting by homologous recombination between DNA sequences residing in the chromosome and newly introduced DNA sequences in pluripotent mouse embryonic stem cells (ES). The aim of the described method is to generate mice of any desired genotype. The document gives an overview with regard to early gene targeting experiments and reports on page 72, left column, that the repertoire of the technique has been extended in 1985, when it was shown that endogenous genes were a suitable subject.

The ability to modify specific chosen sites of the ES cell genome by gene targeting is demonstrated by the example of disrupting the *hprt* gene by insertion of the neomycin resistance gene (*neo*^{R}), which not only disrupts the coding sequence of *hprt* but also acts as a selectable marker. References (1) and (36) quoted in document (24), two publications co-authored by the author of document (24), correspond to documents (16) and (20) respectively in the present proceedings. They also refer to this embodiment of gene targeting and have been cited by the respondents to prove the general availability of methods using homologous recombination techniques. Another publication dealing with exactly the same subject-matter is document (18).

Document (24), on page 73, in figure 3, goes on to discuss the two different kinds of vectors useful for homologous recombination, i.e. sequence replacement and sequence insertion vectors. It is stated (left column, end of second full par.) that "with sequence insertion vectors the entire targeting vector is inserted into the target, thereby automatically transferring both the desired mutation and the selectable gene into the endogenous locus."

Starting on page 75, the final chapter of document (24) is concerned with "Uses of Gene Targeting". It is expected that gene targeting will contribute significantly to neurobiology and will provide mouse models for human genetic diseases. The last paragraph of the document reads: "Gene targeting may also provide an additional tool for mapping and/or isolating human genes. Gene targeting can be used to insert dominant selectable genes into specific chromosomal regions.

Once the selectable gene is in place, it can be used to transfer that chromosomal region, via chromosome-mediated gene transfer, into new recipient cells, or to isolate deletions surrounding the selectable gene or to clone DNA sequences into its vicinity."

To summarize, document (24) discloses a second aspect of the claimed method, namely homologous recombination technique, which is used according to the patent in suit to bring the amplifiable gene into the vicinity of the target gene, and to construct in situ an expression cassette in the primary cell.

25. The question to be asked by the board is, whether a skilled person being aware of the closest state of the art, document (17), and trying to solve the problem underlying the patent in suit, i.e. to develop alternative techniques for producing proteins of interest in culture with cells, would obviously consider to combine the teaching of document (24) with the closest state of the art in such a way as to fall within claim 1. In other words, would it be obvious to change the method of document (17) by replacing co-transformation of a target gene and an amplifiable gene into an expression host by transforming a primary cell comprising a target gene with a construct comprising an amplifiable gene by homologous recombination, followed by the transfer of the complete expression cassette formed in situ in the primary cell into a secondary expression host cell, as set out in claim 1 of the patent?

26. Document (17) on page 168, under the heading "Optimizing Expression of heterologous genes" contains a statement reading: "The level of protein expression from heterologous genes introduced into mammalian cells depends on multiple factors including DNA copy number, efficiency of transcription, mRNA processing, mRNA transport, mRNA stability and translational efficiency, and protein processing, secretion and stability. The rate limiting step for high-level expression may be different for different genes. Controls at each one of these levels will be discussed in turn."

This statement teaches that high level expression of heterologous proteins of interest is a complex task whose success depends on multiple factors.

Document (17), on page 186, lines 1 to 5, addresses the problem of the patent in suit: "This chapter has primarily focused on the various approaches to high-level expression of proteins in a variety of mammalian cell systems. Future developments will involve the modification of mammalian cells in order to increase the efficiency of the various steps in protein processing and secretion."

Thus, the document opens a broad range of possible avenues of further research for the skilled man trying to provide an alternative to already existing techniques. Read in the light of the passages on pages 168 and 186, as cited above, document (17) does not provide the skilled reader with any input prompting him to modify the coamplification technique described on pages 170 to 174, being one out of a number of possible ways to optimize gene expression only.

27. The skilled person is far from being in a "one-way-situation" leading him to combine the closest state of the art with document (24), but rather he is faced with various alternatives to solve the problem.

Document (24), as well as all other cited documents reporting on homologous recombination events (see point (24) above), does not refer to protein expression, thus belonging to a different, though neighbouring technical field. There are references to gene targeting using dominant selectable markers, but none envisages the use of selectable markers being amplifiable genes as well, nor the possible technological benefits possibly resulting therefrom. The final paragraph of document (24), although opening the window for future applications of homologue recombination technology, must merely be considered as an invitation addressed to the skilled reader for doing further research.

Therefore, at the day of filing of the patent in suit it was not obvious for a skilled person, trying to solve the posed problem, to change the method for high level expression of proteins disclosed in the closest prior art, document (17), by the introduction of a homologous recombination step, based on the disclosure in document (24), or other cited documents, which are concerned with gene targeting in mouse embryonic stem cells, and to transfer the expression cassette formed thus in situ into a secondary expression host cell.

28. For the same reasons an objection to claim 1 under Article 56 EPC, based on document (24) as closest state of the art, must fail.

29. Following another line of argumentation, the inventive concept of claim 1 was questioned based on the statement in decision T 939/92 (OJ EPO 1996, 309), referring to broad claims in the field of chemistry, saying that in view of the state of the art a technical effect which is the sole ground for an alleged inventive step had to be achieved by all embodiments falling within the scope of a claim (see 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.

Respondents II, for two reasons, argued that the method of claim 1 did not solve the posed problem, by not providing a method "for economic and efficient production of the desired protein". However the problem here was to find an alternative method. This was done and the alternative was not obvious. The alternative cannot be said to be inherently uneconomic or inefficient, and so the problem can be regarded as solved.

30. According to the case law of the Boards of Appeal, for an inventive step to be present in claims referring to the alternative solution of a known problem, it is not necessary to show substantial or gradual improvement over the prior art (decision T 588/93, 31 January 1996).

31. Consequently, the board comes to the conclusion that the subject-matter of claim 1 allows to achieve a technical effect over its whole scope which solves the problem underlying the invention.

32. Dependent claims 11 and 12, referring to the use of DHFR deficient secondary host expression cells, were attacked as not solving the problem underlying the invention. Respondents II argued on the basis of the disclosure in document (11) that an amplification system using DHFR deficient cells did not work.

The board does not agree. The authors of document (11), being aware of the limited applicability of the DHFR amplification system, were merely concerned with the development of an alternative system based on the adenosine deaminase (ADA) cDNA gene (see page 3136, left column). The document contains no statement from which it can be concluded that a system for amplification and selection of heterologous genes using DHFR deficient CHO cells does not work.

33. The conclusions drawn with regard to an inventive step of claim 1 apply in the same way to independent claim 7. For that reason, claims 1-13 meet the requirements of Article 56 EPC.

Order

For these reasons it is decided:

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 claims of auxiliary request 1 filed at the oral proceedings on 8 May 2003 with the description and drawings as granted.

The Registrar: The Chairwoman:

P. Cremona U. M. Kinkeldey