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Datasheet for the decision of 9 April 2021

Case Number: T 1904/15 - 3.3.08

Application Number: 02791720.2

Publication Number: 1453966

IPC: C12P21/02, C12N15/85, C07K14/505, C12N15/90

Language of the proceedings: EN

Title of invention:
Method for producing a recombinant polypeptide

Patent Proprietor:
Sandoz AG

Opponents:
Strawman Limited

Headword:
Transfection method/SANDOZ

Relevant legal provisions:
EPC Art. 54, 56, 84, 123(2)
RPBA 2020 Art. 12(2), 12(3)
RPBA Art. 12(4), 13

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It can be changed at any time and without notice

Keyword:
Main request filed at the oral proceedings - admission - (yes)
Main request filed at the oral proceedings - requirements of the EPC complied with - (yes)

Decisions cited:
T 0301/12, T 1566/12

Catchword:

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Case Number: T 1904/15 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 9 April 2021

Appellant: (Opponent 02)	Strawman Limited Orchard Lea Horns Lane Combe, Witney Oxfordshire OX29 8NH (GB)
Representative:	Eliot Ward Mewburn Ellis LLP Aurora Building Counterslip Bristol BS1 6BX (GB)
Respondent: (Patent Proprietor)	Sandoz AG Lichtstrasse 35 4056 Basel (CH)
Representative:	Wegner-Cribbs, Natalia Carpmaels & Ransford LLP One Southampton Row London WC1B 5HA (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 6 July 2015 concerning maintenance of the European Patent No. 1453966 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. Montrone
	D. Rogers

Summary of Facts and Submissions

I. The appeal lies against the decision of an opposition division to maintain the European patent No. 1 453 966 in amended form. The patent was filed under the PCT and published as international patent application WO 03/046187 (hereinafter the "patent application").

II. The opposition division held that the main request filed during oral proceedings complied with the requirements of the EPC.

III. Opponent 01 withdrew its opposition with the letter dated 9 January 2015.

IV. With its statement of grounds of appeal, opponent 02 (hereinafter "appellant") made submissions on the issues of added subject-matter, lack of novelty and inventive step concerning the subject-matter of the claims as maintained by the opposition division, and submitted a new document, D33. As regards insufficiency of disclosure, the appellant merely stated that "*We maintain our arguments from the opposition proceedings on this ground*".

V. In reply, the patent proprietor (hereinafter "respondent") filed auxiliary requests 1 to 6, which were new to the proceedings.

VI. In reply, the appellant maintained its arguments on added subject-matter, lack of novelty and inventive step against the main request, and raised objections under added subject-matter, lack of clarity and inventive step against auxiliary requests 2 to 6.

VII. In further submissions, the parties provided counter-arguments to each other's case. *Inter alia*, the respondent offered to replace the term "*are*" in claims 7 of auxiliary requests 5 and 6 by "*were*" to overcome a lack of clarity objection raised by the appellant.

VIII. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's provisional, non-binding opinion.

IX. In reply, the parties provided further arguments, and the appellant announced that it would not be represented during the oral proceedings to be held by videoconference.

X. Oral proceedings before the board were held on 9 April 2021 by video conference as requested by both parties. During the oral proceedings, the respondent withdrew the main request as maintained by the opposition division, and auxiliary requests 1 to 6, filed in reply to the appellant's statement of grounds of appeal, and submitted a new main request.

XI. Claims 1, 7 and 11 of the main request submitted on 9 April 2021 read as follows:

"1. A method for producing a transformed eukaryotic host cell which expresses a recombinant polypeptide of interest which method comprises simultaneously introducing into a eukaryotic host cell:

(a) a first polynucleotide vector which comprises (i) a first nucleotide sequence which encodes a recombinant polypeptide of interest, and (ii) a second nucleotide sequence encoding a selectable marker, which second

nucleotide sequence is amplified when the host cell is contacted with a selection agent, and

(b) a second polynucleotide vector having the same nucleotide sequence as the first polynucleotide vector except that the second nucleotide sequence is replaced with a third nucleotide sequence which encodes a different selectable marker;

the first polynucleotide vector and second polynucleotide vector being integrated into the genome of the host cell, which method further comprises adapting the host cell to serum-free medium.

7. A transformed eukaryotic host cell comprising, integrated into one or more chromosomes:

(a) a first polynucleotide vector which comprises (i) a first nucleotide sequence which encodes a recombinant polypeptide of interest; and (ii) a second nucleotide sequence encoding a selectable marker, which second nucleotide sequence is amplified when the host cell is contacted with a selection agent and

(b) a second polynucleotide vector having the same nucleotide sequence as the first polynucleotide vector except that the second nucleotide sequence is replaced with a third nucleotide sequence which encodes a different selectable marker;

wherein the first and second polynucleotide vectors were simultaneously introduced into the host cell and wherein the host cell has been adapted to serum-free medium.

11. A method for producing a recombinant polypeptide of interest which method comprises culturing a transformed eukaryotic host cell, for example a CHO cell, in serum-free medium under conditions that permit expression of the first nucleotide sequence, wherein the host cell comprises, integrated into one or more chromosomes:

(a) a first polynucleotide vector which comprises (i) a first nucleotide sequence which encodes a recombinant polypeptide of interest; and (ii) a second nucleotide sequence encoding a selectable marker, which second nucleotide sequence is amplified when the host cell is contacted with a selection agent and

(b) a second polynucleotide vector having the same nucleotide sequence as the first polynucleotide vector except that the second nucleotide sequence is replaced with a third nucleotide sequence which encodes a different selectable marker.

wherein the first and second polynucleotide vectors are simultaneously introduced into the host cell."

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

D7: EP 0 319 206 (published 7 June 1989);

D8: WO 89/10959 (published 16 November 1989);

D12: WO 89/00605 (published 26 January 1989);

D13: US 5,420,019 (published 30 May 1995);

D16: J. Sambrook and D. Russel, Molecular Cloning, 2001, 3rd edition, Volume 3, 16.47;

D22: K. Maruyama *et al.*, Methods in Nucleic Acids Research, edts. Karam, Chao, Warr, 1991, Chapter 14, 283-305;

D28: G. M. Lee *et al.*, Journal of Biotechnology, 1999, Vol. 69, 85-93;

D33: Declaration of Dr. H. Prentice, dated 10 November 2015.

XIII. The appellant's written submissions, insofar as relevant to the present decision, may be summarised as follows:

Admission into the proceedings of a claim request, a ground of opposition, and document D33

Auxiliary requests filed in reply to the appellant's statement of grounds of appeal should *inter alia* not be admitted into the proceedings because they diverged from the main request as maintained by the opposition division and lacked clarity.

Insufficiency of disclosure as a ground of opposition formed part of the present appeal proceedings. This was so because the summons to oral proceedings were issued before 1 January 2020, the date when the Rules of Procedure of the Boards of Appeal 2020 (RPBA 2020) came into force (hereinafter the "date"). According to the transitional provisions of Article 25(1) RPBA 2020, in general, the RPBA 2020 rules apply to appeals pending on that date. However, Article 25(2) RPBA 2020 provides that Article 12(4)-(6) RPBA 2020 shall not be applied to statements of grounds filed before that date, and Article 25(3) RPBA 2020 provides that Article 13(2)

RPBA 2020 shall not be applied to cases where the summons to oral proceedings had been notified before that date, but instead that Article 13 RPBA 2007 shall continue to be applied. In other words, while Article 12(1), (2) and (3) RPBA 2020 applied to the present case, Articles 12(4)-(6) and 13(2) RPBA 2020 did not. Article 12(1) RPBA 2020 states that the appeal proceedings shall be based on *inter alia* the decision under appeal and the minutes of the oral proceedings before the department having issued the decision. Since parts 6.1 and 6.2 of the decision under appeal, and points 29 to 31 of the minutes presented the appellant's arguments on insufficiency, these arguments formed part of the present appeal proceedings.

Main request

Added subject-matter

The deletion of the term "*essentially*" in paragraph [0036] of the patent in suit from the definition of "~~*essentially*~~ *"the same" means that the sequences of the vectors, with the exception of the selectable marker sequences, are identical or nearly so*" added subject-matter. This was so because the deletion allowed for a broader interpretation of "*the same*" throughout the description of the patent compared to the patent application which disclosed on page 8, fifth paragraph the more limited definition "*essentially the same*". This broader interpretation of "*the same*" applied, for example, to paragraph [0051] of the patent which stated "*It is nonetheless convenient to use vectors that are identical outside of the second/third nucleotide sequences because in practice, <u>the same</u> vector is used as the basis of cloning the first and second vector*" (emphasis added). Since according to amended

paragraph [0036] of the patent "*the same*" meant now also "*nearly so*", paragraph [0051] of the patent encompassed the use of vectors that were similar but non-identical outside the marker genes, for example, in their cloning sites. However, the use of these vectors was excluded from the corresponding paragraph on page 11, fifth paragraph of the patent application when read in conjunction with page 8, fifth paragraph of the patent application which defined "*the same*" as identical.

Inventive step

The claimed invention lacked an inventive step, in light of the teaching of document D7 when combined with document D12; in light of document D8 when combined with document D7; in light of document D12 alone; or in light of document D13 alone, or when combined with document D28.

Document D7 disclosed the generation of transformed eukaryotic cells expressing a recombinant protein of interest, and its production in these cells. This was achieved by a supertransfection, i.e. co-transfection of host cells (CHL-1 and CHL-2) by two different vectors (pPA003 and pPA208 or pPA209), both expressing the same gene encoding a protein of interest and a different marker gene, one of which was amplifiable (see Example II, Figure 1A). Document D12 disclosed the same vector pair (pPA003 and pPA209, see Figures 1B and 1D) as document D7, and that the single transfection of the CHL-1 host cell with the pPA525 vector instead of pPA003 resulted in an increased recombinant protein production: 0.870 vs 0.20-0.35 mU/cell/day (see page 32, line 10 and 11, Table I on page 44 of document D12). Based on this information in document D12, the

skilled person would have returned to document D7 to replace pPA003 by pPA525 in transfecting CHL-1 cells to obtain CHL-2 cells and to supertransfect these with the pPA209 vector to arrive at host cells transfected with the vector pair pPA525 and pPA209. These two vectors differed in their selection marker genes only, one being amplifiable (dihydrofolate reductase (DHFR), see Figures 1A and 1B of document D12), i.e. were the same as required by claims 1, 7 and 11. Moreover the vectors were integrated into the chromosomes (see page 13, lines 11 to 15 of document D12). The only feature lacking when combining documents D7 and D12 was the adaptation of the host cell to serum-free conditions, which was trivial, and hence, obvious.

Document D8 disclosed likewise transformed host cells and their use in a method for the production of proteins of interest. Starting from document D8, the skilled person was taught that recombinant genes integrated into the genome could be amplified in host cells co-transfected by two vectors with different selection markers, one of them being amplifiable (see page 13, lines 21 to 25, page 27, lines 1 to 22). The vector pair used in document D8 (pPA003 and pPA509/510, see Figure 1) contained three selection markers, and was, hence, less similar, when compared to the vector pair in document D7 (pPA003 and pPA209/210, see above) with two markers only. Although cells co-transfected with the **less** similar vector pair disclosed in document D8 achieved a higher protein production than cells co-transfected with the **more** similar vectors disclosed in document D7 (see document D7, Table II on page 13 and Table IV B on page 15), there was no disincentive derivable from this teaching to use the more similar vectors of document D7. This was so because the teaching in document D8 went further than the claimed

invention by showing that the use of multiple markers led to better results than single markers. This teaching was independent from the identity of the vector sequences outside the markers and, hence, did not teach away from using the vectors in document D7.

As regards document D12, according to claim 7 of the main request and in view of paragraph [0031] of the patent, there was no requirement that both vectors as defined in steps (a) and (b) were completely integrated into the chromosomes. In other words, the integration of vector parts was sufficient, for example, one copy of the gene encoding a protein of interest and one copy of each marker gene. Document D12 disclosed a pPA208-transfected cell with an integrated amplifiable marker, a selectable marker, and an integrated gene encoding a protein of interest (see page 40, lines 29 to 35). Document D12 therefore only differed from the claimed invention in the adaptation of the transfected host cell to serum-free conditions. Since this was known to be desirable, the claimed subject-matter was obvious for the skilled person.

Document D13 disclosed a further method for increasing the production of recombinant proteins in eukaryotic host cells, and the generation of a transformed host cell. Starting from Example 2C of document D13, the integration of the two co-transfected vectors into the genome was at least to some degree inevitable. Further the claimed invention did not require that the host cell was adapted to serum-free medium in an absolute sense. It was sufficient that during the generation process such an adaptation took place. Example 2C mentioned that between the two transfection steps minimal medium devoid of serum was used for culturing the cells (see column 15, line 59 to column 16, line

9). This implied that the cells in Example 2C were adapted to these conditions. Even if Example 2C did not disclose adapted cells, other working examples in document D13 suggested their use (see e.g. Example 2A, column 14, lines 49 to 53, Example 2E, column 17, lines 31 to 33), or it was obvious from the teaching of document D28.

The patent did not contain any evidence of a surprising technical effect that was ascribable to the features distinguishing the claimed subject-matter from documents D7, D8, D12, or D13. In particular, the patent did not contain evidence in support of an increased amplification rate, a high protein production, or an enhanced chromosomal stability. Nor have comparative data in support of such effects been submitted. Accordingly, the technical problem to be solved was the provision of an alternative method of expressing a cloned gene product in a transformed eukaryotic cell in which expression vectors each containing different selectable markers and an expression cassette encoding the same structural gene have been integrated into the genome of the transformed eukaryotic cell.

The most obvious alternative was the production and use of vectors having the same nucleotide sequence except the marker genes, i.e. the use of "similar" vectors. This was the simplest, most conventional and routine approach for the skilled person to take (see, for example, document D22, Figure 6C). The overall simplification of the claimed subject-matter resulting therefrom was therefore obvious.

XIV. The respondent's submissions, insofar as relevant to the present decision, may be summarised as follows:

Admission into the proceedings of a new main request, a ground of opposition, and document D33

The main request was essentially based on auxiliary request 5 filed in reply to the appellant's statement of grounds of appeal. The amendments in this auxiliary request were admissibly filed in reply to the notices of opposition (see auxiliary request 4 submitted with the letter dated 2 December 2013), and merely brought in line with the amendments of the main request found allowable by the opposition division during the first instance proceedings. Therefore, auxiliary request 5 was submitted at the earliest possible opportunity. Furthermore, the term "*are*" in claim 7 of auxiliary request 5 was replaced by "*were*" to arrive at the new main request. This amendment was suggested in the letter dated 19 January 2018 to overcome the appellant's objection under lack of clarity against auxiliary request 5. It was straightforward, and did not raise any complex issues. Hence, the new main request should be admitted into the proceedings.

Document D33 should not be admitted into the proceedings (Article 12(4) RPBA). It provided firstly comments on document D13 in relation to a novelty attack, i.e. for a document that has been present since the onset of the opposition proceedings. Secondly, it commented on technical effects of the patent which had been put forward by the respondent likewise during the written stage of the opposition proceedings.

Main request

Added subject-matter

The deletion of the term "*essentially*" from paragraph [0036] of the patent did not add subject-matter, since when compared to the corresponding fifth paragraph on page 8 of the patent application, the amendment did not introduce a new technical teaching.

Inventive step

Document D8, but not D13 represented the closest prior art for the subject-matter of claims 1, 7 and 11. It differed at least in three features from the subject-matter of these claims, while document D13 differed in five features. The simultaneous transfection of a eukaryotic host cell by a first and a second vector allowed for a simpler and faster generation of transformed cells to be used for producing recombinant proteins, since the selection for two markers was carried out in a single step, that did not require two successive rounds of selection, separated by several weeks. This led to the provision of an improved method and means for the production of recombinant proteins. This problem was solved by the subject-matter of claims 1, 7 and 11.
Furthermore, the claimed solution to this problem was not obvious for the skilled person starting from either of documents D8 or D13, since none of these documents provided any pointers to the features of the claims. In particular, none of the available documents hinted at the simultaneous transfection of a cell by two vectors to be used for the production of proteins of interest. While simultaneous co-transfection of vectors into a host cell belonged to the common general knowledge of the skilled person, as for example, derivable from document D16, there was no suggestion for its use in the context of the claimed invention. Accordingly, the

skilled person would not have arrived in an obvious manner at the subject-matter of claims 1, 7 and 11.

XV. The appellant requested that the decision under appeal be set aside and that the patent be revoked. It further requested that none of the auxiliary requests be admitted into the appeal proceedings.

XVI. The respondent requested that the decision under appeal be set aside and that the patent be maintained upon the basis of the main request filed during oral proceedings. It further requested that document D33 not be admitted into the proceedings.

Reasons for the Decision

Admission into the proceedings of a new main request

1. The main request submitted at the oral proceedings before the board corresponds to auxiliary request 5 filed in reply to the appellant's statement of grounds of appeal, except that in claim 7 the term "*are*" has been replaced by "*were*".

2. Auxiliary request 5 itself corresponds to the main request found allowable by the opposition division, except that the feature "*simultaneously*" has been added to claim 1, and the feature "*wherein the first and second polynucleotide vectors are simultaneously introduced into the host cell*" has been added to claims 7 and 11. These amendments are derived from claims 1 and 7 of auxiliary request 4 which was submitted in reply to the then opponents 01 and 02 notices of opposition (see respondent's letter dated 2 December 2013). Accordingly, auxiliary request 5 in the appeal proceedings was amended in line with the

main request maintained by the opposition division, and includes a further amendment proposed since the beginning of the opposition proceedings.

3. Since the amendments in auxiliary request 5 have been filed at the earliest possible opportunity by the respondent during the opposition and the appeal stages, the board admitted auxiliary request 5 into the proceedings.

4. The amendment in claim 7 of the main request submitted during the oral proceedings (see point 1, above) was introduced to overcome a clarity objection raised by the appellant in its letter dated 5 September 2017. The amendment is straightforward since it overcomes an internal inconsistency in claim 7, and does not result in any new complex issues.

5. Accordingly, exercising its discretion under Article 13 RPBA 2007, the board decided to admit the new main request into the proceedings. In reply, auxiliary request 5 was withdrawn by the respondent during the oral proceedings (see item X, above).

Admission into the proceedings of a ground of opposition, and document D33

6. In its statement of grounds of appeal, the appellant submitted under insufficiency of disclosure (Articles 83 and 100(b) EPC) merely the following: "*[W]e maintain our arguments from the opposition proceedings on this ground*".

6.1 In a later submission in reply to the board's preliminary opinion, the appellant submitted that insufficiency of disclosure formed a ground of

opposition in the appeal proceedings, because in the present case due to the transitional provisions of Article 25(1) RPBA 2020, Articles 12(4)-(6) and 13(2) RPBA 2020 did not apply, while Article 12(1) RPBA 2020 applied.

6.2 Moreover, since Article 12(1) RPBA 2020 required that the appeal proceedings be based on the decision under appeal and minutes of any oral proceedings before the department that issued this decision, the appellant's arguments on insufficiency presented in parts 6.1 and 6.2 of the decision under appeal, and points 29 to 31 of the minutes formed part of the appeal proceedings.

7. The board is not convinced by these arguments. While the provisions of the RPBA 2020 referred to by the appellant above apply to the case, the assessment of whether or not insufficiency forms part of the proceedings as a ground of opposition is not limited thereto. Further provisions of the RPBA have to be taken into account, including, that of Articles 12(2) and (3) RPBA 2020 and Article 12(4) RPBA 2007.

7.1 Article 12(1) RPBA 2020 requires *inter alia* that appeal proceedings are based on (a) the decision under appeal and minutes of any oral proceedings before the department having issued that decision; and (b) the notice of appeal and statement of grounds of appeal filed pursuant to Article 108 EPC.

7.2 Moreover, Article 12(2) and (3) RPBA 2020 set out that in view of the primary object of the appeal proceedings to review the decision under appeal in a judicial manner, a party's appeal case has to be directed to the requests, facts, objections, arguments and evidence on which the decision under appeal was based. For that

reason, the statement of grounds of appeal and the reply shall contain a party's complete case. These submissions should set out clearly and concisely the reasons why a party requests that the decision under appeal be reversed, amended or upheld, and should specify expressly all the requests, facts, objections, arguments and evidence relied on.

7.3 In the present case, these requirements are not fulfilled by a passing reference in the statement of grounds of appeal to the appellant's arguments put forward in the opposition proceedings. It is not for the board to identify issues which arose in opposition proceedings and may (or may not) still be a matter of dispute in appeal proceedings, but for the appellant to put forward in the statement of grounds of appeal its line(s) of argument(s) and each of the facts and evidence on which it relies in appeal proceedings. According to the case law, a mere reference to submissions made at first instance, as a rule, cannot replace an explicit account of the legal and factual reasons for the appeal (see Case Law of the Boards of Appeal of the EPO, 9th edition 2019 (hereinafter "Case Law"), V.A.2.6.4., 1173 and V.A.3.2.1.5.j), 1195; *inter alia*, T 1566/12, Reasons point 20, and T 301/12, Reasons points 29 to 31).

7.4 In line therewith, the ground of opposition under Articles 100(b) and 83 EPC does not form part of the present appeal proceedings.

8. The appellant further submitted document D33 in its statement of grounds of appeal.

8.1 According to Article 12(4) RPBA 2007, the board has a discretion to hold inadmissible facts, evidence or

requests which could have been presented in the first instance proceedings. In this context, the board may *inter alia* take account of whether or not a convincing case has been made as to why the document could not have been filed earlier.

8.2 Document D33 is an expert declaration by Dr. Holly Prentice referring in its first part (see points 2 to 8) to Example 2C of document D13, which has been filed with the appellant's notice of opposition.

8.3 In its second part (see points 9 to 16), document D33 refers to the patent in suit, in particular to Examples 5, 9 to 12, and paragraphs [0057], [0076], [0178], [0188] and [0190], and addresses the issue whether or not these passages support a high recombinant protein expression and chromosomal stability of the transformed host cells mediated by the claimed methods. These beneficial effects have been pointed out by the respondent (then patent proprietor) in reply to the notices of opposition (see letter of 2 December 2013, points 5.9 and 5.10).

8.4 Thus, the appellant submitted document D33 to contest effects in the patent and provide arguments in support of document D13 for the first time at the appeal stage. However, the patent and document D13 are known to the appellant since the beginning of the opposition proceedings, and reasons why document D33, as an attempt to provide counter-arguments or further support, could not have been submitted in the first instance proceedings are not apparent to the board. Nor have reasons been submitted by the appellant.

8.5 In view of these considerations, document D33 is not admitted into the appeal proceedings.

Added subject-matter of the patent description

9. The appellant argued that the deletion of the term "*essentially*" from the definition in paragraph [0036] of the patent reading "~~*essentially*~~ *"the same" means that the sequences of the vectors, with the exception of the selectable marker sequences, are identical or nearly so*" (emphasis added) added subject-matter. This was so because the deletion of the limiting "*essentially*" allowed a broader interpretation of "*the same*" as "*nearly so*", while in the patent application "*the same*" meant "*identical*" only.

10. The board is not convinced by the appellant's argument. Amended paragraph [0036] of the patent reads as follows: "*The vectors according to the invention possess sequences which are similar apart from the selectable markers. The first selectable marker is an amplifiable gene; the second need not be amplifiable. As used herein, "the same" means that the sequences of the vectors, with the exception of the selectable marker sequences, are identical or nearly so. In practice, the two vectors are typically based on the same starting vector, in which the selectable marker gene is replaced. Thus, the remaining sequences will be Identical, save for any spontaneous changes which may occur. Advantageously, the sequences, excepting the marker genes including their respective control regions, are more than 85% identical; advantageously, 90% identical; and preferably at least 95%, 98%, 99% or even completely identical*" (emphasis added).

11. This paragraph of the patent corresponds to the fifth paragraph on page 8 of the patent application. Both paragraphs are identical, except that the patent

application uses in the third sentence the term "*essentially the same*" instead of "*the same*".

12. The definition of the term "*the same*" as being "*identical or nearly so*" has not changed in amended paragraph [0036] of the patent, when compared to the respective paragraph in the patent application which defines "*essentially the same*" as being "*identical or nearly so*". In both paragraphs the terms refer to all sequences of the vectors of the invention, except the selectable marker genes. In other words, while the selectable marker genes are different between the vectors, the remaining sequences of the vectors are either identical or nearly identical, which includes any potential cloning sites. This can be derived from the first, the fourth and the fifth sentence in both paragraphs, which further indicate that in practise typically "*the same starting vector*" (i.e. parental vector) is used, and that in the final vectors "*the selectable marker gene is replaced*". The remaining sequences in the vector "*will be Identical, save for any spontaneous changes which may occur*" (i.e. vectors that are "*nearly*" the same, including, for example, modified cloning sites).

13. The definition of "*the same*" in paragraph [0036] of the patent is in line with paragraph [0051] of the patent, which likewise refers to "*the same vector is used as the basis of cloning the first and second vector*" (emphasis added), i.e. the same parental vector. Thus, the patent application, like the patent, relates to vectors of the invention which are identical or nearly so in all sequence parts except their marker genes. This identity or near identity encompasses in both documents the multiple cloning sites. Hence, paragraph [0036] of the patent does not contain subject

matter extending beyond the content of the application as filed.

14. The requirements of Article 123(2) EPC are fulfilled.

Main request (filed during oral proceedings of 9 April 2021)

Claim construction - claims 1, 7 and 11

15. The subject-matter of claims 1, 7 and 11 requires that:

15.1 (i) a first and a second vector are "*integrated*" into the genome of a eukaryotic host cell (hereinafter the "**integration**" feature). In other words, the transformed eukaryotic host cells according to claims 1, 7 and 11 are characterised by having integrated in their genomes the two vectors defined in steps (a) and (b). This requires that at least the vector elements specified in steps (a) and (b) are integrated into the genome, i.e. two copies of a gene encoding a polypeptide of interest and one copy of an amplifiable and a selectable marker gene. In view thereof the board does not agree with the appellant's submission that the claims merely require the integration of one copy of the gene encoding a protein of interest, in addition to the two marker genes.

15.2 (ii) step (a) defines that the second nucleotide sequence in the first vector encodes a selectable marker which "*is amplified when the host cell is contacted with a selection agent*" (hereinafter the "**amplifiable**" feature). It is established case law that features in a claim have to be given their broadest technically sensible meaning (see Case Law, I.C.4.1, 113). Step (a) is silent on any amplification step, and merely mentions that this takes place "*when*" the cell

"*is contacted*" with a selection agent. It is further uncontested that the gene amplification can be carried out at any stage following the integration of an "amplifiable" marker into the genome. The board is therefore not convinced by the respondent's arguments that the term "*amplified*" in step (a) necessarily implies that gene amplification of the marker has indeed occurred. It suffices that the selectable marker is "amplifiable" in the presence of a suitable selection agent.

15.3 (iii) the features "*the second polynucleotide vector having the same nucleotide sequence as the first polynucleotide vector except that the second nucleotide sequence is replaced with a third nucleotide sequence which encodes a different selectable marker*" (emphasis added, hereinafter the "**similarity**" feature) recited in step (b) specifies that the two vectors contain at least two marker genes that are different, while the remaining vector sequences including the genes encoding a protein of interest are identical. This feature, however, does not limit the vectors to those which differ from each other solely by the marker genes *per se*, since these genes may form functional units with control regions (see paragraphs [0012] and [0036] of the patent). Furthermore, the term "*replaced*" does not impose further restrictions on the claimed subject-matter, for example, the location and orientation of the different markers in the vectors.

15.4 (iv) step (b) in claims 1, 7 and 11 is silent on the amplification of the second marker gene. Thus, step (b) is directed to selectable markers that may be amplifiable or not.

15.5 (v) the claims are further directed to a method for producing eukaryotic host cells that undergo an adaptation process to serum-free medium (see claim 1), to cells being adapted thereto (see claim 7), and a method for producing a recombinant protein in serum-free medium (see claim 11) (hereinafter the **"adaptation"** feature). The board interprets the adaptation step in claim 1 to result in host cells being adapted to serum-free medium. Since none of the claims define the time point of adapting the cells, it may take place prior, in between, concomitant with, or after the cell's transfection with both vectors. Although claim 11 refers to a culturing step of host cells in serum-free medium without mentioning adaptation, the expression of a gene of interest under these conditions necessarily implies that the cells are adapted to this medium too.

15.6 (vi) the claims are further characterised in that the host cells are simultaneously transfected with the two vectors defined in steps (a) and (b) (hereinafter the **"co-transfection"** feature). This excludes methods where the transfection of two vectors into a host cell takes place in two successive, i.e. timely separated transfection rounds. Furthermore, it is textbook knowledge that "*At some point within the first few hours after transfection, the incoming DNA undergoes a series of nonhomologous intermolecular recombination and ligation events to form a large concatemeric structure that eventually integrates into the cellular chromosome. Each transformed cell usually contains only one of these packages, which can exceed 2 Mb in size (Perucho et al. 1980). Stable cell lines can then be isolated that carry integrated copies of the transfected DNA. Transformation rates vary widely from cell type to cell type. In the best cases, ~ 1 cell in*

10^3 in the original transfected population stably expresses a gene(s) carried by the transfected DNA." (see document D16, page 16.47, first paragraph). In other words, two vectors with two different selection markers that are simultaneously transfected into a cell integrate at the same site into the chromosome, because the vectors form a "*large concatemeric structure*" due to intracellular linearisation and ligation events.

Added subject-matter, clarity and novelty

16. The appellant has not raised objections under added subject-matter against any of the claim requests. The board has none either. The main request thus complies with Article 123(2) EPC.

17. The appellant has raised a clarity objection against claim 7 of *inter alia* auxiliary request 5, on which the present main request is based. By changing the tense from "*are*" in claim 7 of auxiliary request 5 to "*were*" within the feature "*wherein the first and second polynucleotide vectors were simultaneously introduced into the host cell*" in claim 7 of the main request, this objection is overcome. Other objections under lack of clarity were not raised by the appellant, nor by the board. Thus the main request complies with Article 84 EPC.

18. The appellant has not raised any novelty objections against auxiliary request 5 on which the present main request is based. The board has none either. Accordingly, the main request complies with Article 54 EPC.

Inventive step

Closest prior art

19. It is contested between the parties which document(s) represent(s) the closest prior art. The appellant submitted that any of documents D7, D8, D12 and D13 represented the closest prior art, while the respondent selected document D8.

20. According to established case law, a document aiming at the same purpose or effect and having most of the relevant technical features in common with the claimed invention normally represents the closest prior art (see Case Law, I.D.3.1).

21. Document D7 discloses a gene amplification system for the expression of gene products at increased levels in transformed eukaryotic host cells (see abstract). Example II on page 13, line 30, discloses the host cell CHL-2 which is transfected with the plasmid pPA 003 that comprises the genes encoding the tissue plasminogen activator (t-PA) as the protein of interest and neomycin (neo) as the selection marker (see for pPA 003, page 10, lines 32 to 35, Figure 1A). The CHL-2 cells are then supertransfected, i.e. successively co-transfected, with the vectors pPA 208 or pPA 209, both comprising the t-PA gene, but dihydrofolate reductase (dhfr) as an "amplifiable" selection marker instead of neo (see page 13, lines 30 to 39, Figure 1A). The transfection of CHL-2 cells with two vectors instead of one increases the production of t-PA (see Table II on page 13). The plasmids pPA 208 and pPA 209 differ from pPA 003 in the marker gene (dhfr instead of neo), and in that the dhfr expression cassette contains in addition an SV40 T intron and a fragment of the

tetracycline (tet) resistance gene (see Figure 1A and its Legend). Example II further reads on page 13, lines 40 to 42: "*This example demonstrates that plasmids with the dhfr gene in opposite orientations can both function as dominant selection markers, and that this selection may also be done in CHL-2 cells which contain an endogenous dhfr gene.*" The reference to plasmids in this passage implies that the vectors are not integrated into the genome. Example II of document D7 is thus silent on the "integration", "adaptation", and the "co-transfection" features recited in claims 1, 7 and 11. Moreover, the "similarity" feature is not disclosed, since the dhfr expression cassette contains additional sequences aside from the marker and its control elements (see above).

22. Document D8 reports as primary object the provision of methods and plasmids that lead to a high production of recombinant polypeptides (see page 21, lines 14 to 16). Gene amplification is one of the parameters to be considered in this context (see page 27, lines 9 to 20). Document D8, like document D7, discloses CHL-2 cells containing the plasmid pPA 003. These cells are successively supertransfected with the plasmids pPA 509 or pPA 510 (see page 26, line 10 to page 27, line 8). All plasmids express recombinant t-PA, but pPA 509/510 differ from pPA 003 by a fragment of the tet resistance gene and a SV40 T intron, and by having two selectable markers (the "amplifiable" dhfr and hygromycin (hmb), instead of one (neo)) (see page 22, lines 8 to 10, page 25, lines 19 to 25 and Figure 1). Page 13, lines 21 to 25 of document D8 reads: "*After transfection into eukaryotic cells, the plasmids will either integrate into the host's genome or remain extrachromosomally replicating. In the embodiment exemplified, the plasmids described integrate into the host*

cell" (emphasis added). Contrary to the respondent's view, the board considers that this passage in document D8 directly and unambiguously discloses that the exemplified plasmids, i.e. pPA 003, pPA 509 and pPA 510, integrate into the host cell's genome, since the term "*integrate*" in the second sentence, although not mentioning the integration site, can only be understood to refer back to the first sentence which specifies the site as the genome. Document D8 is, however, silent on the "adaptation", the "similarity" (see Figure 1), and the "co-transfection" features of claims 1, 7 and 11.

23. Document D12 likewise reports methods for increasing the expression of recombinant genes for the production of polypeptides at high levels in eukaryotic host cells (see abstract, page 1, lines 8 to 21, page 6, lines 10 to 12). Document D12 mentions that the transfected vectors may be integrated into the host cell's genome or not (see page 13, lines 11 to 13). The document further discloses on page 40, line 10 to page 41, line 4 that the orientation of the dhfr gene relative to the t-PA gene affects t-PA's transcription level in cells transfected with a **single** vector having two selection markers (dhfr and neo). Thus, document D12 discloses a transfection of host cells by a single vector carrying both marker genes, and not by two vectors, a concept fundamentally different from the one claimed. Furthermore, document D12 is silent on the "adaptation" feature recited in claims 1, 7 and 11.

24. Document D13 discloses in Example 2C (see column 15, line 37 to column 16, line 35) a strategy to achieve an optimal expression of desired recombinant "*rBPI products*" (bactericidal/permeability-increasing proteins). Mammalian Sp2/O cells (see column 13, lines 12, 13, 25 and 26) (i) are transfected with the

expression vector "*pING42**23***" (emphasis added) having a rBPI gene and the marker gene "*gpt*" (xanthine-guanine phosphoribosyl transferase mediating a mycophenolic acid (MPA) resistance), (ii) high producer cells are screened, and (iii) the same cells are then transfected with the vector "*pING42**21***" (emphasis added) containing the same rBPI gene as the first vector, but "*his*" as the marker gene conferring resistance to the selection agent histidinol (see for the construction of pING4221 and pING4223, column 7, line 44 to column 8, line 12). The patent in suit discloses in paragraph [0034] that gpr genes, a synonym of the gpt gene in Example 2C of document D13, belong to "*Common amplifiable systems*", that "*are amplifiable as such in that the nucleic acid is itself amplified in response to selection pressure applied by cognate agents*". Hence, the "amplifiable" feature of step (a) of claims 1, 7 and 11, for the reasons set out above (see point 15.2), is disclosed in document D13.

24.1 Document D13 further states in column 8, lines 4 to 10: "*Similar constructions were made exactly as described for pING4223 except that different SstII-SalI vector fragments were used to generate vectors with different selection markers. For example, pING4221 is identical to pING4223 except that it contains the his marker (conferring resistance to histidinol) instead of gpt*" (emphasis added). The SstII-SalI vector fragment has a size of about 8000 bps (see column 7, lines 38 to 42). In other words, the sequences of the pING4221 and pING4223 vectors are identical, except for a 8000 bp long SstII-SalI fragment containing the gpt or the his selection markers.

The respondent submitted that the reported length of 8000 bps in document D13 was too long for the marker

gene and its control elements. Thus it was likely, that the fragment contained further non-marker sequences which resulted in vectors not sharing the "similarity" feature of claims 1, 7 and 11.

The board does not agree. Firstly, the marker genes of the vectors in claims 1, 7 and 11 are not limited to a particular size, and secondly, the passage in column 8 of document D13 states "*pING4221 is identical to pING4223 except that it contains the his marker (conferring resistance to histidinol) instead of gpt*" (emphasis added). This corresponds to the wording in step (b) of claims 1, 7 and 11 which states: "*a second polynucleotide vector having the same nucleotide sequence as the first polynucleotide vector except that the second nucleotide sequence is replaced with a third nucleotide sequence which encodes a different selectable marker*" (emphasis added). Moreover, since the "similarity" feature is not limited to the marker genes but includes regulatory sequences (see above), it is disclosed in Example 2C of document D13.

24.2 The appellant has submitted that Example 2C of document D13 further discloses adaptation to serum free medium, i.e. the "adaptation feature" of claims 1, 7 and 11. The board does not agree. Example 2C mentions that the Sp2/O cells are maintained in DMEM medium supplemented with 10% FBS (fetal bovine serum) before being transfected with the first vector. After transfection, the cells are recovered in non-selective DMEM medium for 48 hours, followed by incubation in DMEM medium supplemented with the selection agent MPA for 1.5 to 2 weeks for obtaining clones expressing rBPI. These cells are then transfected with the second vector, and recover in DMEM medium supplemented with 10% FBS followed by incubation in the same medium for 1.5 to 2

weeks to obtain high producer cell clones (see column 15, lines 51 to column 16, line 21). Since a DMEM medium with 10% FBS is not serum-free, the cells transfected with both vectors cannot be regarded as being adapted to serum-free medium.

24.3 The appellant has further submitted that Example 2C of document D13 disclosed that both vectors are integrated into the transformed host cells' genomes, i.e. the "integration" feature. The board does not agree. Example 2C reports in column 15, lines 53 to 55 that the plasmid pING4223 "*had been digested with NotI*", i.e. linearised before being transfected into Sp2/O cells. The finding of transformed host cells as described in lines 63 to 66 of column 15 necessarily implies that the first vector has been integrated into the genome. However, no such vector linearisation prior to transfection is mentioned for the second vector pING4221 (see column 16, lines 10 to 12). The appellant argued that also the second vector was integrated into the genome, because the reported time period of 1.5 to 2 weeks in Example 2C for obtaining transfected clones was identical after the first and the second transfection step (see column 15, line 65, and column 16, line 18). This is not convincing. Column 4, lines 2 and 3 of document D13 reports that "*Also provided are autonomously-replicating DNA plasmid vectors*", i.e. episomal vectors. It is common general knowledge that episomal vectors, even in the absence of a selection agent, stably transfect host cells, and hence, express recombinant proteins (see e.g. document D22, page 290, last paragraph). Thus, the reported rBPI expression and secretion from transformed cells in Example 2C (see column 16, lines 22 to 35) allows no direct and unambiguous conclusion about the potential integration

of the rBPI gene into the genome, since this could likewise be due to the presence of an episomal vector.

24.4 In summary, Example 2C of document D13 is silent on the "integration", "adaptation", and the "co-transfection" features of claims 1, 7 and 11.

25. Consequently, all of documents D7, D8, D12 and D13 are directed to the same purpose underlying the claimed subject-matter, namely the generation or provision of transformed eukaryotic cells expressing a recombinant protein of interest, or the production of a recombinant protein of interest by these cells. The methods/host cells disclosed in documents D8 and D13 share most of the relevant technical features with the claimed subject-matter (since they differ in three features only), while document D7 differs in four features, and document D12 relates to a different concept (transfection of one vector instead of two).

26. In line with the case law, the board considers that documents D8 and D13 represent the closest prior art for the subject-matter claimed. The claimed subject-matter differs from that disclosed in documents D8 and D13 in that these documents do not disclose the "adaptation" and the "co-transfection" features. Document D8 further differs in the "similarity" feature, while document D13 differs in the "integration" feature.

27. As regards the technical effects ascribable to these distinguishing features the following is noted:

27.1 The adaptation to serum free medium allows an improved large-scale production of recombinant proteins, because this avoids problems caused by serum components in down

stream process steps (see e.g. document D28, page 86, column 1, second paragraph).

27.2 The "co-transfection" feature , i.e. the simultaneous transfection of host cells by both vectors, simplifies and speeds up the generation of transformed host cells, and hence, the production of proteins from these cells, by reducing the necessary transformation and selection steps.

27.3 Although the respondent submitted that the "similarity" feature is associated with various beneficial effects, such as increased protein production yield, chromosomal stability, and gene amplification rate, including a simplified vector construction, the board is not convinced by these arguments. Firstly, the patent lacks any evidence that the claimed method results in increased recombinant protein production, chromosomal stability and gene amplification. Comparative data between the claimed vector systems and those of documents D8 or D13 have also not been submitted. Furthermore, the construction of the transformation vectors is not a feature that falls within the scope of claims 1, 7 and 11.

27.4 The "integration" feature leads to stably transformed host cells showing a constant protein production. Document D22, which is an excerpt from a textbook, reports that episomal vectors likewise allow a stable transfection of host cells (see page 290, last paragraph). A potential benefit of integrated genes vs genes on episomal vectors is therefore not apparent.

28. In light of these considerations, only the effects associated with the "adaptation" and "co-transfection" features are considered for the formulation of the

technical problem, but not those submitted for the "similarity" or the "integration" feature, since they are either not supported by data or unrelated to the claimed subject-matter.

29. Accordingly starting from documents D8 or D13, the technical problem may be formulated as the provision of an improved method and means for the production of recombinant proteins.

30. This problem is solved by the subject-matter of claims 1, 7 and 11 in view of the working examples provided in the patent.

31. It remains to be assessed whether or not the skilled person starting from document D8, or from Example 2C of document D13 and faced with the technical problem identified above, would have arrived at the claimed subject-matter in an obvious manner.

32. Although document D8 does not suggest adaptation to serum free medium for the production of recombinant proteins, it suggests the production of various therapeutic proteins, including the protein tissue plasminogen activator (t-PA) (see page 15, lines 10 to 16, and lines 29 and 30). In the board's view, the skilled person would derive from document D8 that it is directed at the production of therapeutic human proteins. Therefore the skilled person would consider the cultivation of the host cells in serum-free medium to simplify down-stream industrial production processes. Accordingly, the adaptation to serum free medium in the method of document D8 is considered obvious for the skilled person. However, as regards a simultaneous vector transfection, document D8 deals exclusively with the transfection of a host cell in a

successive manner, i.e. a host cell is first transfected with a first vector, followed by the selection and isolation of transformed clones showing the required properties. In a second round, the selected cells are then transfected with the second vector, followed by another round of selection and isolation. Document D8 provides no pointers to transfect the host cell simultaneously with both vectors. Accordingly, the subject-matter of claims 1, 7 and 11 is not obvious in the light of the teaching of document D8 alone.

33. The same considerations as regards the simultaneous transfection apply, if the skilled person starts from Example 2C of document D13. Concerning the adaptation to serum free medium, document D13 explicitly suggests this in several working examples for protein production purposes (see e.g. column 17, lines 31 to 33 or lines 55 to 57). Consequently, the subject-matter of claims 1, 7 and 11 is also not obvious in the light of the teaching of document D13 alone.

34. It is uncontested that the simultaneous transfection of host cells by different vectors belongs to the common general knowledge of the skilled person (see e.g. document D16, page 16.47, second paragraph). Despite this fact, documents D8 or D13 neither rely on the simultaneous transfection nor provide pointers thereto to further simplify and speed up the generation of transformed host cells for the production of recombinant proteins. While the skilled person could have used simultaneous transfection to solve the above mentioned technical problem, the board is not convinced that the skilled person would have done so in the absence of any pointers. Therefore, the subject-matter of claims 1, 7 and 11 is not considered obvious in the

light of the teaching of documents D8 and D13 when taking common general knowledge into account.

35. The "co-transfection" feature is also not disclosed in documents D7 or D28, which were cited by the appellant in combination with either documents D8 or D13, respectively as closest prior art. Accordingly, even if the teaching of both documents would be combined, the skilled person would not arrive at the subject-matter of claims 1, 7 and 11.

36. Thus, the main request complies with Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is remitted to the opposition division with the order to maintain the patent on the basis of claims 1 to 17 of the main request received during the oral proceedings of 9 April 2021, and a description to be adapted thereto.

The Registrar: The Chairman:

H. Jenney B. Stolz

Decision electronically authenticated