Datasheet for the decision
of 12 November 2008

Case Number: T 0665/07 - 3.3.08
Application Number: 94913175.9
Publication Number: 0695361
IPC: C12N 15/90

Language of the proceedings: EN

Title of invention:
Expression of heterologous genes according to a targeted expression profile

Patentee:
THE UNIVERSITY COURT OF THE UNIVERSITY OF EDINBURGH

Opponent:
Institut Pasteur

Headword:
Heterologous genes/EDINBURGH

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

Relevant legal provisions (EPC 1973):
-

Keyword:
"Main request: added-matter (yes)"
"Auxiliary request 1: added-matter (yes)"
"Auxiliary request 2: inventive step (no)"
"Auxiliary request 3: added-matter (no)"
"Sufficiency of disclosure (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
-
Catchword: -
Case Number: T 0665/07 – 3.3.08

DECISION
of the Technical Board of Appeal 3.3.08
of 12 November 2008

Appellant: Institut Pasteur
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Composition of the Board:
Chairman: L. Galligani
Members: T. J. H. Mennessier
C. Rennie-Smith
Summary of Facts and Submissions

I. The opponent (appellant) lodged an appeal against the decision of the opposition division dated 6 February 2007, whereby the opposition filed against the European Patent No. 0 695 361 was rejected under the provisions of Article 102(2) EPC 1973.

II. The patent with the title "Expression of Heterologous Genes According to a Targeted Expression Profile" was granted on European application No. 94 913 175.9, which was filed as an International application under the PCT on 21 April 1994, published as WO 94/24301.

III. The patent was opposed on the grounds as set forth in Article 100(a) EPC that the invention was neither new nor inventive, Article 100(b) EPC that it was not sufficiently disclosed and Article 100(c) EPC that the application had been amended in such a way that it contained added matter.

IV. The statement of grounds of appeal was filed on 15 June 2007. The patent proprietor (respondent) did not reply.

V. A communication under Article 15(1) of the Rules of Procedure of the Boards of Appeal dated 7 August 2008 presenting some preliminary and non-binding views of the board was then sent to the parties.

VI. The respondent replied to the board's communication with a letter dated 13 October 2008. It requested that the patent be maintained as granted (main request) or on the basis on one of the four auxiliary requests filed with its letter.
Main request (claims as granted)

Claim 1 read:

"1. A method of inserting a heterologous gene coding sequence into a target endogenous gene in a eukaryotic cellular host cell genome and expressing said heterologous gene coding sequence, by transforming the host cell with a vector comprising a DNA construct, characterised in that the host cell is selected from a non-human embryonic stem cell and a non-human fertilised egg, and in that the DNA construct comprises the sequence:

\[ 5' \text{X-A-P-B-Q-C-Y} 3' \]

in which

X and Y are substantially homologous with separate sequences from the target endogenous gene and are of sufficient length to undergo homologous recombination with the host cell genome so as to insert the A-P-B-Q-C sequence into the host cell genome;

P is an internal ribosome entry site (IRES);

Q is the heterologous gene coding sequence; and

A, B and C are, separately, optional linker sequences,

wherein the construct inserts the heterologous gene coding sequence \textbf{into or in place of} the target endogenous gene so that transcription of the heterologous gene coding sequence is directed by the
host regulatory elements for the target endogenous gene." (emphasis added by the board)

Claims 2 to 11 were dependent on claim 1 and directed to specific embodiments thereof.

Claim 12 read:

"12. A method of inserting a heterologous gene coding sequence into a eukaryotic cellular host cell genome comprising the steps of:

(i) random integration of a first DNA construct into the genome; and

(ii) homologous recombination of a second DNA construct into the genome using the method of any of Claims 1-11, wherein the random integration step comprises expressing said coding sequence under control of elements regulating expression of an endogenous gene in a donor cell genome, said donor cell being a different cell from said host cell, by allowing the first DNA construct to undergo random integration into the host cell genome, wherein the host cell is selected from a non-human embryonic stem cell and a non-human fertilised egg and the first DNA construct comprises the sequence:

5' X'-A'-P'-B'-Q'-C'-Y' 3'

in which

X' and Y' are substantially homologous with separate sequences from the same donor cell genome and comprise the elements
regulating expression of the endogenous
gene in the donor cell;
P' is an internal ribosome entry site
(IRES);
Q' is the heterologous gene coding
sequence; and
A', B' and C' are, separately, optional linker
sequences."

Claims 13 to 18 and 20 were dependent on claim 12 and
directed to particular embodiments thereof.

Claim 19 read:

"19. A method according to any of Claims 1-17, wherein
the host cell is selected from a mouse embryonic stem
cell and a mouse fertilized egg."

Auxiliary request 1 consisted of 20 claims and differed
from the main request in that (a) the word
"heterologous" as used for the second time in the
preamble of claim 1 as granted ("and expressing said
heterologous gene coding sequence" [emphasis added])
has been deleted, (b) the word "sequence" after "the
A-P-B-Q-C" has been replaced by the word "elements" and
(c) the expression "and in the same respective
orientation as in the endogenous locus" was added both
in claim 1, after "from the target endogenous locus" to
further characterise the sequences X and Y, and in
claim 12, after "from the same donor cell genome" to
further characterise the sequences X' and Y'.

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Auxiliary request 2 consisted of 12 claims corresponding to claims 1 to 11, as well as claim 19 made dependent thereon, of auxiliary request 1.

Auxiliary request 3 consisted of 12 claims and differed from auxiliary request 2 only in that, in the last paragraph of claim 1, the expression "or in place of" was no longer present (see claim 1 as granted supra).

VII. On 13 October 2008, third party's observations were filed under the provisions of Article 115 EPC.

VIII. With a fax-letter dated 20 October 2008, the appellant informed the board that it withdrew its request for oral proceedings and that it was not intending to attend the oral proceedings.

IX. Oral proceedings which took place on 12 November 2008 were attended only by the respondent.

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


(D6) WO 90/11354 (published on 4 October 1990)
XI. The submissions made (in writing) by the appellant, insofar as they are relevant to the present decision, may be summarised as follows:

Main request (claims as granted)

Article 123(2) EPC

The expression "heterologous gene coding sequence" was introduced into the claims during the examination proceedings in place of the expression "heterologous gene sequence". The two expressions did not cover the same product. Indeed, the first one designated only the coding sequences of a heterologous gene. The only place in the application as filed (see the published version WO 94/24301) where insertion of a coding sequence was discussed (see page 10, first full paragraph) stated that the invention permitted a heterologous coding sequence to be inserted into the 3' untranslated region of a gene. As claim 1 was not so limited, it contained subject-matter which extended beyond the content of the application as filed.

Claim 1 which had no counterpart in the claims as filed specified that the sequences X and Y were substantially homologous with separate sequences from the target endogenous gene but failed to indicate that those sequences should also be in the same respective orientation as in the endogenous locus (see page 7, second paragraph in the application as filed).

There was no support in the application as filed for a two step method according to claim 12, in which use was made of two different DNA constructs to perform a
random integration and a homologous recombination in that order.

The method of claim 9 which added to the steps of the method of claims 1 to 8 an **identification** step of the cells expressing the heterologous gene coding sequence had no support in the application as filed. Such a step was not described therein. None of the terms "isolation", "detection" and "selection" used throughout the description as filed covered the same concept as that encompassed by the term "identification" which includes the idea of "individualisation".

**Article 83 EPC**

The embodiment of claim 1 involving the insertion of the DNA construct **in place of** the target endogenous gene was not sufficiently disclosed.

**Article 54 EPC**

The patent was not entitled to the claimed priority dates. Therefore, document D1 was relevant for the novelty assessment. This document, to which two of the inventors contributed, disclosed the outline of the experiments of Example 1 of the patent. Thus, the method of claim 1 lacked novelty.

**Article 56 EPC**

In view of document D6 which represented the closest state of the art, the technical problem solved by the invention was regarded as the provision of a method
which permitted the functional integration of a heterologous gene coding sequence into a desired endogenous gene transcribed region without producing a fusion protein while avoiding the need of disrupting endogenous gene expression (see the paragraph bridging pages 8 and 9 in the application as filed). The skilled person, facing that technical problem of replacing part of a coding sequence by a heterologous gene (see from page 8, line 34 to page 9, line 2), would have inevitably used an IRES, as such a sequence was well-known at the relevant filing date of the patent (see document D3) to permit the independent translation of a given gene on the same transcription unit as another gene. Therefore, claim 1 lacked an inventive step.

Moreover, the "in place" embodiment of claim 1 lacked an inventive step for the reason that, as stated in the application as filed (see page 12, second full paragraph), the IRES was optionally omitted.

Auxiliary requests

The appellant did not take position on the auxiliary requests as such.

XII. The submissions made by the respondent, insofar as they are relevant to the present decision, may be summarised as follows:

Main request (Claims as granted) - claim 12
(Article 123(2) EPC)
The last paragraph on page 12 of the description as filed (see the published version WO 94/24301) and the first paragraph on page 13 provided adequate basis for claim 12, when read with the second and third paragraphs of page 13. These passages described an insertion method for use when the host cell genome lacked a suitable endogenous gene target for homologous recombination. The specification described a method in which random integration of a first DNA construct into a cell, in which a target endogenous gene having a suitable expression profile was not present or accessible, was used to create a modified cell where there was a suitable gene to target via homologous recombination. Thus, random integration of the first DNA construct into the cell resulted in a modified cell which was a target for DNA constructs according to any embodiment of the invention operating via homologous recombination (see page 13, third paragraph).

The prime symbol used to distinguish each of the components of the first DNA construct indicated that those components might be the same or not as the corresponding components of the DNA construct of claim 1. With reference to page 12 of the description as filed, the component X' was a gene "H" of the donor cell which had the same expression profile as the gene "G" of the target host cell.

Auxiliary request 1

The remarks made in respect of Article 123(2) EPC regarding claim 12 of the main request applied similarly to claim 12 of auxiliary request 1.
Auxiliary request 2

The use of an IRES in the DNA constructs used to perform the "in place" embodiment of claim 1 provided the advantage that the preparation of the construct was made easier, as there was no longer any need to select a precise place in the construct for the ATG codon to be used as the starting point of the translation. This improved the efficiency of the claimed method. Therefore, that embodiment was inventive and thus complied with the requirements of Article 56 EPC.

Auxiliary request 3

Article 123(2) EPC

The application as filed discussed constructs comprising promoter regions derived from one gene fused to cDNA coding sequences from another gene (see page 2, final paragraph) or the use of cDNA coding for the heterologous protein of interest (see page 3, end of second full paragraph). Although such prior art methods had significant effects, it would nevertheless be apparent that, when expressing a heterologous protein under the direction of the host regulatory elements of the target endogenous gene (as required in claim 1), the heterologous gene would be provided without its regulatory elements (i.e. the coding sequence would be used). This was also made clear at page 13, final paragraph in the application as filed, which described the constructs of the invention as "promoterless constructs". Such promoterless constructs did not include the whole heterologous gene sequence. Rather
they contained the coding sequence of the heterologous gene.

Claim 1 incorporated the wording "and in the same respective orientation as in the endogenous locus". The appellant's objection raised against claim 1 as granted in this respect was moot.

As indicated in the board's communication of 7 August 2008, the word "identifying" used in claim 9 was intended to express that the skilled practitioner performing the method of claim 9 would have to determine which of the cells he/she was manipulating had successfully incorporated in their genome the heterologous gene of interest and were capable of expressing it. In that context, the use of the word "identifying" was not objectionable.

**Article 83 EPC**

In claim 1 the "in place" embodiment had been deleted. Therefore, the appellant's objection raised against claim 1 as granted in this respect was also moot.

**Article 54 EPC**

The expression "heterologous gene coding sequence" was presented in the same general terms in the latest priority document (GB 94 010 011.3 filed on 20 January 1994). The expression was present at page 7, second full paragraph and page 9, first paragraph of the document. Therefore, claim 1 was entitled to the priority date of 20 January 1994. As a result document D1, which was published in February 1994, was not part
of the state of the art. As this was the only document referred to by the appellant as being novelty-destroying, claim 1 was new.

Article 56 EPC

There would have been no incentive for the skilled person to add an IRES, as described in document D3, in the DNA constructs of document D6. Thus, claim 1 involved an inventive step.

XIII. The appellant requested that the decision under appeal be set aside and the patent be revoked.

XIV. The respondent requested that the appeal be dismissed or that the patent be maintained on the basis of one of auxiliary requests 1 to 4 filed on 13 October 2008.

Reasons for the Decision

Main request (claims as granted)

1. Claim 12 has been objected to under Article 123(2) EPC. It is directed to a method of inserting a heterologous gene coding sequence into a eukaryotic, cellular host cell genome. The method comprises a step of random integration of a first DNA construct into the genome followed by a step of homologous recombination of a second DNA construct into the genome using the method of claims 1 to 11.

2. The first DNA construct is stated to comprise the sequence 5' X'-A'-P'-B'-Q'-C'-Y' 3', in which X' and Y'
are substantially homologous with separate sequences from the same donor cell genome and comprise the elements regulating expression of the endogenous gene in the donor cell, P' is an IRES and Q' is the heterologous gene coding sequence. The said formula as such is not found in the application as filed. What is described in the application as filed (see the passage bridging pages 12 and 13) is a DNA construct which includes "(1) the cell D regulatory elements for a targeted endogenous gene, the expression profile E of which is desired to be mimicked, (2) an IRES and (3) a heterologous gene sequence G". As stated in the preceding sentence, the cell D regulatory elements are contained in X and Y. Without any further guidance in the application as filed, the skilled person can only conclude that X is not the same as X', G is not the same as Q' and Y is not the same as Y', and that thus the DNA construct of the random integration step of claim 12 is not the same as the DNA construct described on pages 12 and 13 of the application as filed. Thus, claim 12 contains added matter and consequently the claims as granted as a whole do not comply with the requirements of Article 123(2) EPC.

Auxiliary request 1

3. Claim 12 of auxiliary request 1 has been objected to under Article 123(2) EPC for exactly the same reason as claim 12 of the main request. Therefore, the conclusion reached at point 2 supra applies in the same way. Claim 12 contains added matter and consequently auxiliary request 1 as a whole does not comply with the requirements of Article 123(2) EPC.
Auxiliary request 2

4. Claim 1 of auxiliary request 2 covers as regards the insertion of the heterologous gene coding sequence two embodiments, the sequence being inserted either into the target endogenous gene or in place thereof (the "in place of" embodiment).

5. According to page 12, second full paragraph in the application as filed, in case of the "in place of" embodiment, "the IRES is optionally omitted". In other words, the IRES is admittedly unnecessary.

6. As accepted by the respondent, document D6 represents the closest prior art. According to an aspect, it describes a method of inserting a heterologous gene coding sequence (referred to as "l'ADN d'insertion") into a target endogenous gene in a eukaryotic cellular host cell genome, using a DNA construct comprising the sequence to be inserted flanked by two sequences which correspond to the sequences of the host genome referred to as the "ADN de complement" and are capable of homologous recombination therewith. The regulatory sequences of the endogenous gene are contained within the "ADN de complement". Upon homologous recombination, the newly inserted gene coding sequences are under the control of the host regulatory elements (promoter) for the target endogenous gene (see page 4, lines 28 to 36; page 5, lines 1 to 19; page 6, lines 21 to 29; and page 7, lines 28 to 31). According to an embodiment of the method, the complete coding sequence of the endogenous gene is replaced by the inserted sequence (see the sentence bridging pages 8 and 9).
7. Thus, the method of claim 1, insofar as the "in place of" embodiment is concerned, differs from the method of document D6 essentially in that the DNA construct of claim 1 comprises an IRES. The IRES being the component of the DNA construct which contributes to the art, an inventive step assessment should focus on the question whether that contribution was inventive. As the IRES is unnecessary, the "in place of" embodiment of the method of claim 1 does not contribute anything to the art and an inventive step cannot be acknowledged for that embodiment. Therefore, claim 1 lacks inventive step and auxiliary request 2 does not comply with the requirements of Article 56 EPC.

Auxiliary request 3

8. Auxiliary request 3 consists of 12 claims which correspond to claims 1 to 11 and claim 19, made dependent thereon, as granted, with two amendments in claim 1, namely the introduction of the expression "and in the same respective orientation as in the endogenous locus" after "from the target endogenous locus", to characterise further the sequences X and Y, and the deletion of "or in place of".

Requirements of Article 123(2) EPC

9. These amendments remedy two of the defects objected to under Article 123(2) EPC by the appellant regarding claim 1 as granted. It remains to be assessed whether the amendment of the expression "heterologous gene sequence" to "heterologous gene coding sequence" has introduced added matter.
10. The first full paragraph of page 8 in the application as filed, when considered together with the paragraph bridging pages 9 and 10 describes the basic concept of the invention, i.e. the insertion of a construct containing the coding sequence of a heterologous gene, including or not the introns (see further Example 1, page 24, lines 2 and 3, in the application as filed) into a host endogenous gene in such a way that the transcription of those sequences is under control of the regulatory elements (promoter; see the two first sentences of the paragraph bridging pages 13 and 14 of the application as filed) associated with the host gene. Those pages provide the required support for present claim 1. Thus, introduction of the term "coding" into the expression "heterologous gene sequence" has not resulted in the presence of added matter. Therefore, claim 1 complies with the requirements of Article 123(2) EPC.

11. A further objection of added matter, associated with the word "identifying", has been made with respect to claim 9 as granted and dependent claims 10 and 11 (insofar as those later claims refer back to claims 9 or 10). That objection applies also to claim 9 of auxiliary request 3 as both claims have the same wording. In this respect, the board considers that the word "identifying" intends to express that the skilled person performing the method of claim 9 would have to determine which of the cells he/she is manipulating have successfully incorporated in their genome the heterologous gene of interest and are capable of expressing it. In that context, the use of the word "identifying" is not objectionable under Article 123(2) EPC. The other objections of added matter made by the
appellant were in relation to claims 12 to 18, 19 (insofar as the claim was dependent on any of claims 12 to 17) and 20. As these claims are no longer present in auxiliary request 3, there is no need for the board to consider the issue. The board is satisfied that no other objections of added matter have to be made with respect to auxiliary request 3, which therefore complies with the requirements of Article 123(2) EPC.

Requirements of Article 83 EPC

12. Claim 1 as granted has been objected to for insufficiency of disclosure only with respect to the "in place of" embodiment. As claim 1 of auxiliary request 3 does not contain this embodiment, the objection does not apply thereto. Therefore, the method of present claim 1 is sufficiently disclosed. Thus, auxiliary request 3 complies with the requirements of Article 83 EPC.

Requirements of Article 54 EPC

13. In support of its objection of lack of novelty made against claim 1 as granted, the appellant has relied on document D1 which provides a summary of part of the experiments described in the patent (see in particular the first and second full paragraphs on page 24 of WO 94/24301). The objection, if valid, would apply equally to claim 1 of auxiliary request 3.

14. Document D1 was published on February 1994, i.e. earlier than the date of filing (21 April 1994) of the patent in suit but later than its latest priority date (30 January 1994). Thus, for document D1 to be regarded
as part of the state of the art according to Article 54(2) EPC, claim 1 would have to be found not entitled to any of its three claimed priority dates.

15. The latest priority document with the filing date of 30 January 1994 (to be referred to below as document P3) differs in some respects from the application as filed. It lacks some explanatory passages which are found on pages 1, 4, 5, 9, 14, 15 and 16 of the description as filed (see the passages thereof now referred to in the patent specification as paragraphs 0003 (partly), 0014, 0015, 0016 (partly), 0033, 0034, 0050, 0051 (partly) and 0054 (partly)). Furthermore, its claims 1 to 15 are not directed to methods of inserting a heterologous gene (coding) sequence but to DNA constructs for inserting the same. An equivalent for claim 21 as filed is also lacking.

16. Nevertheless, on pages 5 (from the second full paragraph), 6, 7 and 8 (first line) of document P3 a method as defined in claim 1 of auxiliary request 3 is described with a complete disclosure of the DNA construct including the feature "and in the same respective orientation as in the endogenous locus" (see document P3, page 6, second full paragraph). A disclosure of the host cell being either an embryonic stem cell or a fertilised egg is also provided by document P3 (see Example 1 on pages 16 to 23 and Example 3 on page 23, respectively).

17. Therefore, claim 1 of auxiliary request 3 finds an unquestionable support in the description of document P3. Thus, claim 1 of auxiliary request 3 is entitled to the third priority date (20 January 1994) and
18. As document D1 is the only document used in support of a novelty objection, it is concluded that claim 1 is new. Since claims 2 to 12 are dependent thereon, auxiliary request 3 as a whole complies with the requirements of Article 54 EPC.

Requirements of Article 56 EPC

19. Claim 1 of auxiliary request 3 is directed to a method of inserting a heterologous gene coding sequence into a target endogenous gene in a eukaryotic cellular host cell genome and expressing said gene coding sequence, by transforming the host cell with a vector comprising a particular DNA construct. The construct inserts the heterologous gene coding sequence into the target endogenous gene so that transcription of the heterologous gene coding sequence is directed by the host regulatory elements of the target endogenous gene. The construct contains an IRES which is placed in the direction 5'->3' before the heterologous gene coding sequence.

20. Document D6, which is considered to represent the closest state of the art and describes a method of inserting a heterologous gene coding sequence into a target endogenous gene, has been discussed in detail in point 6 (see supra). According to an embodiment of the method, only part of the coding sequence of the endogenous gene is replaced and the formation of fusion proteins is avoided by the insertion of a heterologous
sequence which begins at the initiation codon of the replaced gene (see the sentence bridging pages 8 and 9).

21. Thus, the method of claim 1 differs from the method of document D6 essentially in that the DNA construct of claim 1 comprises an IRES which is intended to permit the independent translation of the heterologous gene coding sequence.

22. In view of document D6, the technical problem may be regarded as the provision of an alternative method which permits the integration of a heterologous gene coding sequence into a desired endogenous gene transcribed region without producing a fusion protein and without the need to disrupt endogenous gene expression (see the paragraph bridging pages 8 and 9 in the application as filed). The solution to that problem is the method of claim 1.

23. The appellant has argued that the skilled person facing the technical problem would have been prompted by document D3 to introduce in the DNA constructs of document D6 an IRES upstream of the heterologous gene coding sequence and, thus, would have arrived at the invention without the exercise of inventive skill.

24. Therefore, the question to be answered is whether, as contended by the appellant, document D3 would have provided such an incentive.

25. Document D3 reports that a pHR1 vector incorporating an EMCV leader-neo cassette has been designed which can be used to class-switch immunoglobulin heavy-chain genes. The encephalomyocarditis virus (EMCV) leader sequence
which functions as an Internal Ribosome Entry Site (IRES) is responsible for efficient, cap-independent initiation of translation. The pHR1 vector contains a targeted insert made, in the direction 5' to 3', of a 3.55 kb fragment consisting of the human γ1 constant region sequence, the EMCV leader, the neo marker gene, and the SV40 poly(A)-addition signal. The targeted insert is flanked by murine μ sequences that can act as targets for homologous recombination. Upon homologous recombination, the insertion takes place before the murine μ sequences. As a result, not the integrated sequence (human Cγ1) but the endogenous sequence (murine Cμ) is placed under the control of the IRES.

26. This leads to the key observation that document D3 does not contain any guidance as to the concept of a construct in which the sequence to be integrated is placed under the control of an IRES. That concept falls outside the ambit of document D3, the gist of which is the use of a EMCV-neo-cassette to prepare an insert in which the sequence to be integrated is located upstream of the IRES. As a result the skilled person would have found no incentive therein to introduce an IRES in the construct of document D6, in order to get a DNA construct as referred to in claim 1.

27. Thus, the skilled person would not have been in a position to arrive at the method of claim 1 without the exercise of inventive skill. For this reason, claim 1 involves an inventive step, the same conclusion applying de facto to dependent claims 2 to 12. Therefore, auxiliary request 3 as a whole complies with the requirements of Article 56 EPC.
Adaptation of the description

28. An amended description has been provided by the respondent. The amendments were made without going beyond those necessary to adapt the description to the terms of the claims. Thus, they comply with the provisions of Article 123(2) EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of pages 2 to 12 of the amended description filed during the oral proceedings, claims 1 to 12 of auxiliary request 3 filed on 13 October 2008 and the figures as granted.

The Registrar                  The Chairman

A. Wolinski                  L. Galligani