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Datasheet for the decision of 2 February 2023

Case Number: T 1021/21 - 3.3.08

Application Number: 14716610.2

Publication Number: 2986723

IPC: C12N15/00, C12N15/64, C12N15/79

Language of the proceedings: EN

Title of invention:
Plasmid for minicircle production

Patent Proprietor:
Mayrhofer, Peter

Opponent:
PlasmidFactory GmbH & Co. KG

Headword:
Minicircle production/MAYRHOFER

Relevant legal provisions:
EPC Art. 100(a), 56, 100(b)
RPBA 2020 Art. 12(4), 12(6)

Keyword:
Disregard admitted document - (no)
Amendment to case - evidence - (yes)
Inventive step - (yes)
Sufficiency of disclosure - (yes)

Decisions cited:
T 1852/11, T 1525/17

Catchword:
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Case Number: T 1021/21 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 2 February 2023

Appellant: (Opponent)	PlasmidFactory GmbH & Co. KG Meisenstrasse 96 33607 Bielefeld (DE)
Representative:	Grund Intellectual Property Group Patentanwälte und Solicitor PartG mbB Steinsdorfstraße 2 80538 München (DE)
Respondent: (Patent Proprietor)	Mayrhofer, Peter Feistritzgasse 34 1210 Wien (AT)
Representative:	Maiwald GmbH Elisenhof Elisenstraße 3 80335 München (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 4 May 2021 rejecting the opposition filed against European patent No. 2986723 pursuant to Article 101(2) EPC**

Composition of the Board:

Chair	T. Sommerfeld
Members:	B. Claes
	R. Winkelhofer

Summary of Facts and Submissions

I. The appeal lodged by the opponent (appellant) lies from the decision of the opposition division rejecting the opposition against European patent No. 2 986 723 with the title "*Plasmid for minicircle production*". The opposition proceedings were based on the grounds for opposition in Article 100(a), in relation to inventive step (Article 56 EPC), and 100(b) EPC.

Claims 1 and 12 of the patent read:

"1. A plasmid comprising the following units:

- an endonuclease restriction site,
- a promotorless endonuclease expression cassette,
- a promoter,
- a sequence coding for an enzyme that catalyzes site-specific recombination,
- at least two recognition sequences for the enzyme catalyzing site-specific recombination,

and

- a sequence of interest and an element preventing the expression of the endonuclease which are both arranged between said recognition sequences for the enzyme catalyzing site-specific recombination,

wherein the functional units are arranged on the plasmid such that the endonuclease expression cassette is placed under control of the promoter only after recombination at the specific recognition sequences.

12. A method for providing a minicircle, comprising the following steps:

a) transfecting a plasmid according to any of claims 1-11 in an organism capable to replicate said plasmid,
b) recombinating at the recognition sequences for the enzyme that catalyzes site-specific recombination, in order to obtain a minicircle comprising the sequence of interest and the element that prevents the expression of the recombinase and a miniplasmid comprising the endonuclease restriction site, the endonuclease expression cassette, the promoter and the sequence coding for an enzyme that catalyzes site-specific recombination,
c) purifying the minicircle."

Claims 2 to 11 are dependent on claim 1, and claims 13 to 15 are dependent on claim 12.

II. Reference to the Rules of Procedure of the Boards of Appeal (RPBA) in this decision is to the RPBA which entered into force on 1 January 2020, with an amendment in force from 1 April 2021 (OJ EPO 2023, supplementary publication 1, III.2).

III. With the statement of grounds of appeal, the appellant filed arguments that the claimed subject-matter lacked an inventive step and that the patent lacked sufficiency of disclosure for the invention of claims 12 to 15.

IV. The patent proprietor (respondent) replied to the appeal.

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

D1: Chen *et al.*, Hum. Gene Ther., vol. 16, 2005 126-31

D5: WO 2010/002470

D11: Nehlsen *et al.*, Gene Ther. Mol. Biol., vol. 10, 2006, 233-44

D12: Broll *et al.*, J. Mol. Biol., vol. 295, No.5, 2010, 950-65

D13: Broll, Dissertation at the Technical University of Braunschweig, 2009

D15: Conference talk, presented at the XVth Annual Congress of the European Society of Gene and Cell Therapy, Rotterdam, 27-30 October 2007

D16: Conference talk, presented at the XVIth Annual Congress of the European Society of Gene and Cell Therapy, Brugge, 16 November 2008

D17: Heinz *et al.*, "Filing a gap: S/MAR-based replicating minicircles" in The CliniBook, Clinical Gene Transfer State of the Art, O. Cohen-Haguenauer (ed.), Editions EDK/Groupe EDP Sciences, 2012

D28: Davis *et al.*, PLOS Genetics, vol.4, issue 3, e1000028, 2008 (designated as D27 in the statement of grounds of appeal)

D29: Voutev & Hubbard, Genetics, vol. 180, 2008, 103-19 (designated as D28 in the grounds of appeal)

VI. The board summoned the parties to oral proceeding in accordance with their requests and issued a communication under Article 15(1) RPBA by which the parties were informed of the board's preliminary opinion on the admittance of documents D28 and D29 and related arguments in the proceedings on inventive step and sufficiency of disclosure. The board concluded that the appeal would likely be dismissed.

VII. By submission of 27 January 2023, the appellant withdrew the request for oral proceedings and announced that it would not be present if oral proceedings were held. The board then cancelled the oral proceedings.

VIII. The parties' submissions relevant for this decision are discussed in the Reasons below.

IX. The appellant requests that the decision under appeal be set aside and amended such that the patent be revoked.

The respondent requests that the appeal be dismissed and that documents D28 and D29 not be admitted and considered in the proceedings.

Reasons for the Decision

1. The appellant has withdrawn the request for oral proceedings (see section VII.). The decision to dismiss the appeal can thus be taken in writing.

Admittance of document D28

2. The respondent requested that documents D28 and D29 not be admitted and considered in the proceedings.

3. However, the EPC does not provide a legal basis for excluding, in appeal proceedings, documents admitted into the opposition proceedings, particularly if the impugned decision was based on them (see e.g. decisions T 1852/11, Reasons, point 1.3 and T 1525/17, Reasons, point 4.3; Case Law of the Boards of Appeal, 10th edn. 2022, V.A.3.4.4).

Admittance of document D29 and related arguments into the proceedings (Article 12(4) and (6) RPBA)

4. In the statement of grounds of appeal, the appellant makes cursory reference to and arguments on document D29 in the context of inventive step (see points 5.47, 5.55 and 5.56).

5. As is evident from point 4 of the minutes of the oral proceedings and point 6 in the decision under appeal, the opponent dropped its request to have document D29 admitted into the proceedings, and the opposition division in fact announced at the end of the oral proceedings that D29 was not admitted (see point 13 of the minutes).

6. In this procedural situation, Article 12(6) RPBA provides that the board must not admit the evidence. Furthermore, the appellant has not even argued that the circumstances of the appeal case justify its admittance in appeal.

7. Thus, there is no room to admit and consider document D29 in the appeal proceedings.

Inventive step (Articles 100(a) and 56 EPC) - claim 1

8. The invention concerns plasmid-based DNA molecules, so-called minicircle DNA, for use in non-viral gene transfer systems for DNA vaccine design and gene therapy protocols. Minicircle technology aims to remove the bacterial backbone unit from the plasmid upon delivery for gene transfer by an *in vivo* site-specific recombination process. The bacterial backbone unit is needed for the stable propagation of the plasmid-DNA in bacterial cells but is undesired for clinical applications of DNA molecules in humans. By this process, a so-called parental plasmid or maxicircle is divided by a recombinase into a miniplasmid carrying the (undesired) backbone sequences and a minicircle consisting of almost exclusively the desired gene expression cassette (minimal expression cassette).

9. A parental or maxicircle plasmid (see section I) is claimed comprising, in addition to a sequence of interest, an endonuclease restriction site, a promoterless endonuclease expression cassette and a promoter, as well as a sequence encoding a site-specific recombination enzyme and at least two recognition sequences for this enzyme. Between these recognition sequences, the sequence of interest and an element preventing the expression of the endonuclease are included. The functional units are arranged on the claimed plasmid such that the endonuclease expression cassette is placed under control of the promoter only after recombination at the specific recognition sequences on the resulting miniplasmid (which will then be cut at the comprised endonuclease restriction site).

Closest prior art document D1

Difference(s)

10. Document D1 discloses a parental plasmid split into a minicircle and a miniplasmid (see Figure 3A) and represents a suitable starting point for the assessment of inventive step. The sequence of interest in the parental plasmid encodes human factor IX (hFIX) coupled to the eukaryotic sApoE promoter and the bpA terminator and is flanked by recombination sites (attB and attP) recognised by the ΦC31 recombinase. The plasmid includes ΦC31 recombinase encoding sequences coupled to an arabinose-inducible BAD promoter and a sequence encoding an endonuclease (I-Sce1) equally coupled to a BAD promoter.

11. The opposition division identified three differences between the parental plasmid disclosed in document D1 and the claimed parental plasmid: (a) the endonuclease expression cassette was promoterless; (b) the presence of an element which prevented the expression of the endonuclease; and (c) an arrangement of the elements which led to expression of the endonuclease only occurring after recombination (i.e. upon formation of the miniplasmid).

12. Although acknowledging that features (a) and (c) distinguished the claimed parental plasmid from that disclosed in document D1, the appellant argued that the plasmid of document D1 included feature (b) because bpA in the gene of interest cassette (sApoE-hFIX-bpA) was a known transcription terminator and was "preventing the expression of the endonuclease" from the sApoE promoter.

13. The board agrees, however, with the opposition division that the prokaryotic RNA polymerase of *E. coli*, the host cell for the minicircle production, does not bind to the eukaryotic sApoE promoter and cannot therefore lead to any transcription of the endonuclease located downstream which needs to be "prevented". Furthermore, there is also no room for understanding the expression "preventing the expression of the endonuclease" in the claim not to require achievement of complete suppression of expression. Such complete suppression is not achieved within the plasmid of document D1 because regardless of the presence of the bpA termination signal in the expression cassette of the gene of interest, the transcription and expression of the endonuclease are initiated and promoted by the BAD promoter in the endonuclease expression cassette.

14. Accordingly, bpA in the plasmid disclosed in document D1 is not "an element preventing the expression of the endonuclease". The board thus agrees with the opposition division's conclusions on the distinguishing features over D1 (see point 11. above).

Technical effect and problem to be solved

15. The board agrees with the parties and the opposition division that the differences of the claimed plasmid over those of the parental plasmid disclosed in document D1 aim at a temporal separation of the, hence sequential, action of the enzyme mediating the recombination and the action of the endonuclease improving minicircle yield.

16. The opposition division correctly formulated the technical problem as the provision of a plasmid for

producing a minicircle, where the plasmid contains an *alternative* means for separation of the recombinase and endonuclease actions (see decision under appeal, point 14.3). The appellant has not argued differently in the appeal.

17. In view of the positive decision of the board on inventive step for a less ambitious technical problem, the question whether the claimed invention has an *improved* character over the plasmid disclosed in document D1, as alleged by the respondent, can thus be left open.

Obviousness

18. In a first line of argument, the appellant submitted that when starting from the disclosure in document D1, the skilled person would be led to the claimed invention in an obvious manner by the teaching in document D11.

19. Document D11 discloses the production of minicircles from parental plasmids and teaches a plasmid construct for the sequential action of a recombinase and a "sequence under consideration" (a sequence of interest), i.e. EGFP (see Figure 3). The initiation of EGFP action relies on the action of a recombinase. However, in contrast to the situation with the expression activated endonuclease of the claimed plasmid, the promoterless EGFP coding sequence in the parental plasmid is joined to a promoter in the minicircle, but the EGFP expression has no effect on the parental plasmid. Consequently, the EGFP expression correlates with minicircle copy number levels.

20. When the claimed plasmid is transfected into a bacterial cell, the recombinase action increases the copy number level of minicircles, but the endonuclease produced linearises parental plasmids, thus withdrawing substrates from the recombinase action. Consequently, the minicircle copy number level does not correlate with either endonuclease or recombinase expression or activity.

21. Furthermore, neither the parental plasmid nor the host cell used in the method disclosed in document D11 provides expression of an endonuclease, let alone only after recombination has taken place, which can thus also not linearise the produced miniplasmids. In fact, the method of document D11 requires the harvest of total DNA from the host cells.

22. Hence, the board agrees with the opposition division that the claimed plasmid provides for a different mechanism and outcome for minicircle production and endonuclease expression and activity compared to the construct for EGFP expression taught in document D11.

23. The appellant argues in a more general fashion that because document D11 disclosed the principle of a parental plasmid comprising a gene that was initially promoterless but that was brought under the control of a promoter present in the parental plasmid after recombination of the parental plasmid, the skilled person would readily take this idea and apply it to their own needs, i.e. obtaining a protein of interest (endonuclease) at the "destination" of interest (miniplasmid) only active after recombination.

24. This is not persuasive. Indeed, where the construct disclosed in document D11 (see Figure 3A) is such that

recombination joins a promoter to a sequence of interest ultimately found in the minicircle, in the claimed construct, the recombinase action results in joining a promoter to the endonuclease coding sequence (sequence of interest), which after recombination, in contrast, finds itself in the miniplasmid to be linearised and discarded (see point 8. above). Document D11 accordingly suggests to the skilled person to design a construct in which, by action of the recombinase, a promoter is joined to the sequence of interest within the minicircle, this being different from the claimed solution where the sequence of interest ends up in the miniplasmid.

25. Therefore, the opposition division correctly concluded that the appellant's argument that the idea to join a promoter to a promoterless sequence would direct the skilled person to the claimed subject-matter fails.

26. The combination of teachings in documents D1 and D11 thus cannot lead the skilled person in an obvious manner to the subject-matter of claim 1.

27. The alternative combinations of the disclosure in document D1 with that of documents D12, D13 and D15 to D17, which disclose the same parental plasmid construct disclosed in document D11, must equally - for the same reasons - fail to point the skilled person to the claimed solution.

28. In a second line of argument, the appellant submitted that starting from the disclosure in document D1, the skilled person would arrive at the claimed invention in an obvious manner by the teaching in document D28.

29. Document D28 concerns temporally and spatially controlled transgene expression in the eukaryotic nematode *C. elegans*. A construct comprising a promoterless (trans)gene of interest (ORF) preceded by an "off cassette" which prevents functional association of a promoter with this gene of interest and a different plasmid coding for the FLP recombinase are co-injected into *C. elegans*. Recombination results in excision of the "off cassette" as a circular molecule, and this arrangement places the promoter adjacent to the gene of interest, converting this gene of interest to the "on" state (see abstract, Figure 1, page 2, right-hand column, second paragraph). Thus, expression is dependent on both the promoter driving the coding region (ORF) and the promoter driving expression of the recombinase.

30. However, the board agrees with the opposition division that the claimed plasmid is designed to replicate in bacterial culture and further to produce minicircles expressing the gene of interest, which are recombined out of the plasmid by the recombinase action. The production of minicircles is thus a different technical field to the eukaryotic mechanisms disclosed in document D28, where the gene of interest is, in fact, not recombined out of the construct. Therefore, the skilled person would not look in the field of eukaryotic transgene expression to solve the technical problem (see point 15.).

31. Furthermore, the FLP recombinase strategy disclosed in document D28 aims at temporal control of the expression of a gene of interest. This is achieved by an inducible promoter controlling the recombinase expression, which is then linked to the expression of the gene of interest. In contrast, the claimed minicircle strategy

achieves temporal control of the expression of the gene of interest by delaying the endonuclease expression until after the recombinase expression by separating recombinase action and endonuclease expression.

32. Against this backdrop, the claimed subject-matter involves an inventive step in view of the disclosure in document D28.

Closest prior art document D5

33. The appellant also argued that the claimed subject-matter lacks inventive step when starting from the disclosure in document D5 as the closest prior art.

34. Although document D5 discloses a similar parental plasmid construct as document D1, it makes use of different promoters for the recombinase and endonuclease to achieve a temporal segregation of their respective expression. The board also agrees with the respondent that although document D5 defined these promoters as "inducible", the endonuclease coding sequence is functionally linked to a promoter in the parental plasmid, unlike in the claimed plasmid. The assessment of inventive step based on the disclosure in document D5 as the closest prior art therefore mirrors that above for document D1 representing the closest prior art. In fact, as the parental plasmid disclosed in document D5 is similar to that disclosed in D1, the combination of the teachings in document D5 and D11 would also not lead the skilled person in an obvious manner to the claimed subject-matter. The alternative disclosures in documents D12, D13 and D15 to D17 do not point the skilled person to the claimed solution either for the same reasons as for document D11. The board thus agrees with the opposition division that this

alternative approach would not lead to the conclusion that the claimed subject-matter lacks inventive step.

General conclusion on inventive step

35. In view of the above considerations, the subject-matter of claim 1 and its dependent claims involve an inventive step. The same applies to the subject-matter of claims 12 to 15, which are directed to methods relying on the plasmid of claim 1.

Sufficiency of disclosure (Article 100(b) EPC) - claims 12 to 15

36. The appellant submitted that the patent failed to sufficiently disclose the claimed methods because it did not specify the host organism to be used in step a) of claim 12. Thus, any organism was suitable as the host as long as it was capable of replicating the plasmid. Furthermore, although step b) required recombination of the parental plasmid, claim 12 did not provide for the expression of the recombinase on the parental plasmid. Thus, recombination failed if the host organism was not capable of mediating recombination.

37. The board agrees, however, with the opposition division that the microbiologist skilled in the technical field of minicircle DNA production from plasmids is familiar with such plasmids and their replication, transcription and translation in suitable host cells. In fact, paragraph [0080] of the patent discloses several suitable prokaryotic and eukaryotic host organisms and cells for performing the claimed invention, and the examples teach how the invention can be put into practice. Furthermore, the second argument of the

appellant must fail as step a) of the method of claim 12 requires transfecting the plasmid of claim 1 to the host organism, hence providing the sequence coding for a site-specific recombinase.

38. Hence, and also in view of the fact that the appellant has failed to substantiate doubts that the skilled person can work the invention, the patent sufficiently discloses the invention of claims 12 to 15.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

L. Malécot-Grob T. Sommerfeld

Decision electronically authenticated