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D E C I S I O N
of 12 June 2001

Case Number: T 0680/98 - 3.3.4

Application Number: 81301413.1

Publication Number: 0041767

IPC: C12N 15/52

Language of the proceedings: EN

Title of invention:
Improved vectors and methods for making such vectors and for expressing cloned genes

Patentee:
BIOGEN, INC.

Opponent:
BIO-TECHNOLOGY GENERAL CORP.

Headword:
Expression vectors/BIOGEN INC

Relevant legal provisions:
EPC Art. 56

Keyword:
"Main request - inventive step - no"
"Auxiliary request - added subject-matter - yes"

Decisions cited:
T 0301/87, T 0631/89, T 0695/90, T 154/87, T 0426/92

Catchword:
-

Case Number: T 0680/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 12 June 2001

Appellant: (Proprietor of the patent)	BIOGEN, INC. 14 Cambridge Center Cambridge Massachusetts 02142 (US)
Representative:	Jaenichen, Hans-Rainer, Dr. Vossius & Partner Postfach 86 07 67 D-81634 München (DE)
Respondent: (Opponent)	BIO-TECHNOLOGY GENERAL CORP. 1250 Broadway New York (US)
Representative:	Schön, Chr., Dr. Patentanwälte Henkel, Feiler, Hänzel & Partner Möhlstrasse 37 D-81675 München (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted 6 May 1998 revoking European patent No. 0 041 767 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
V. Di Cerbo

Summary of Facts and Submissions

1. The appeal lies from the decision of the Opposition Division dated 6 May 1998 to revoke the European patent No. 0 041 767 with the title "Improved vectors and methods for making such vectors and for expressing cloned genes".

Claims 1 and 6 as granted for all Contracting States except AT read as follows:

"1. A plasmid vector comprising at least one DNA sequence comprising the leftward promoter and operator derived from bacteriophage λ, P_LO_L, said DNA sequence being characterized by the absence of an active cro gene and an active N gene, and by at least one endonuclease recognition site located less than 300 base pairs downstream from P_LO_L in said DNA sequence."

"6. The vector of any one of claims 1 to 5, characterized in that it includes in one of said endonuclease restriction sites a DNA sequence coding for a eukaryotic, prokaryotic or viral protein, polypeptide, enzyme, hormone, antigen or fragment thereof."

Dependent claims 2 to 5 related to further features of the vector of claim 1. Independent claim 7 was directed to a method for producing an improved plasmid vector with the same features as in claim 1, and claims 8 to 11 related to further features of said method. Independent claim 12 was directed to a method for producing a recombinant DNA molecule with the same features as in claim 6. Claims 13 and 14 were directed to methods for producing a polypeptide and claim 15 was directed to deposited plasmid vectors.

The corresponding claims were filed for the Contracting State AT.

II. The Board sent a communication under Article 11(2) of the Rules of Procedure of the Boards of Appeal, stating their preliminary non-binding opinion.

III. In answer to the Board's communication, the Appellants (Patentees) filed a new main request on 11 May 2001. Claim 1 for all Contracting States but AT read as follows:

"1. A plasmid vector characterized by the following features:

(a) the leftward promoter and operator derived from bacteriophage λ, P_LO_L;
(b) the absence of an active <u>cro</u> gene and an active <u>N</u> gene; and
(c) a DNA sequence coding for a eukaryotic or viral protein, polypeptide, enzyme, hormone, antigen or fragment thereof included in an endonuclease recognition site located less than 300 base pairs downstream from P_LO_L ;

wherein said promoter (a) mediates the expression of said DNA sequence (c).

IV. At oral proceedings, the Appellants filed an auxiliary request. Claim 1 of this request read as follows:

"1. A plasmid vector characterized by the following features:

(a) the leftward promoter and operator derived from bacteriophage λ, P_LO_L;
(b) the absence of an active <u>cro</u> gene and an active <u>N</u> gene; and

(c) a DNA sequence coding for a eukaryotic or viral protein, polypeptide, enzyme, hormone, antigen or fragment thereof included in an endonuclease recognition site located less than 150 base pairs downstream from P_LO_L ;

wherein said promoter (a) mediates the expression of said DNA sequence (c) and wherein the vector has less than 150 bp of DNA sequence of bacteriophage λ between P_LO_L and the coding region of the N gene, being located between said promoter (a) and said DNA sequence (c)."

V. The following documents are mentioned in this decision:

(30): Bernard, H-U. and Helinski, D.R., Methods in Enzymology, Vol.68, pages 482 to 492, 1979

(31): Bernard, H-U. et al., Gene, Vol.5, pages 59 to 76, 1979,

(34): Franklin, N.C. and Bennett, G.N., Gene, Vol.8, pages 107 to 119, 1979

(45): Derom, C. et al., Gene, Vol.17, pages 45 to 54, 1982

(46): Remaut, E. et al., Nucleic Acids Res., Vol.11, pages 4677 to 4688, 1983

(55): Horn, G.T. and Wells, R.D., Federation Proc., Vol.38, No.3, Abstract No.383, 1979

(56): Wells, R.D. et al., in DNA-Recombination Interactions and Repair, Eds S.Zadrazil and J.Sponar, Pergamon Press, pages 199 to 210, 1979

(72): Greenfield, L. et al., Biotechnology, Vol. 4, pages 1006 to 1011, 1986

(78): Letter from U. Bernard to E. Remaut filed by the Appellants with their letter dated 16 September 1998

(A3): Declaration by Kinya Ohgami filed with the Respondents' submissions dated 19 April 1999.

VI. The arguments in writing and during oral proceedings by the Respondents (Opponents) which are relevant to this decision can be summarized as follows:

Main request
Admissibility

The Board should disregard the new main claim request as having been filed too late, especially since the Appellants were given an opportunity by the Opposition Division to file a final auxiliary request, which they declined to take advantage of.

Article 84 EPC

- Claim 1 which was derived from granted claim 6 relating, in particular, to DNA sequences encoding molecules of **prokaryotic origin** was neither clear nor supported by the description. Uncertainty stemmed from the fact that the term prokaryotic had been deleted, and that, therefore, it was doubtful whether or not prokaryotic DNA sequences were still comprised within the claim.

- There was no support in the patent specification for vectors comprising DNA sequences coding for eukaryotic proteins inserted less than 300bp from P_LO_L.

Article 56 EPC

- The technical contribution of claim 1 was that the eukaryotic gene to be expressed was positioned less than 300 bp from P_LO_L. No evidence had been provided by the Appellants that this positioning resulted in an improvement of the level of gene expression. On the contrary, the Respondents had shown (document (A3)) that vectors wherein the gene to be expressed was inserted 115 bp or 321 bp downstream from P_LO_L were equally efficient. The levels of expression obtained from these vectors could not be compared to those obtained from vectors wherein the gene to be expressed had been inserted further downstream from P_LO_L (600bp or 1000bp; document (31)) as the amount of protein was not quantified in the same manner.

The experiments described in document (45) whereby a vector according to the patent in suit was used to express the trpA gene also could not be used as evidence for an improved level of expression as they did not comprise a control showing what the level of expression might have been, had the insert been located further downstream from P_LO_L.

The disclosure in document (78) was not sufficient to establish improved gene expression as it lacked experimental details and the author himself questioned the validity of his results.

Post-published document (72) (page 1008) showed that it was not enough to position the gene to be expressed less than 300bp away from P_LO_L to obtain an improved level of expression.

As there was no unexpected technical effect associated with the claimed invention, there was no inventive step.

Auxiliary request
Article 123(2) EPC

The passage of the application as filed cited by the Appellants as providing a basis for the new claim 1 could not be interpreted as a disclosure of the subject-matter of claim 1 ie of a vector which only contained 150 bp of λ DNA between P_LO_L and the coding region of the N gene.

VII. The arguments in writing and during oral proceedings by the Appellants (Patentees) which are relevant to this decision can be summarized as follows:

Main request
Admissibility

The newly submitted main request was in response to the Board's communication and, therefore, in accordance with the practice of the Boards of appeal, it should be admitted into the proceedings.

Article 84 EPC

- The wording of claim 1 was that of granted claim 6 but for the fact that the adjective "prokaryotic" was deleted from the claim. The deletion of one of the claimed embodiments could not give rise to a clarity objection. The skilled person would have no problems in understanding that the adjectives "eukaryotic" and "viral" qualified the products to be expressed.

- The claim did not lack support as the examples provided, even if they dealt with prokaryotic gene expression, would be understood by the skilled person as representative of eukaryotic gene expression.

Article 56 EPC

- The closest prior art was document (31) which disclosed P_LO_L expression vectors where the gene to be expressed was inserted some 600bp or 1000bp downstream from the regulatory region. It was stated on page 74 of said document: "*It is reasonable to assume that genes inserted into the HpaI restriction site upstream from the three sites used in this study will be transcribed with similar efficiency.*"

Starting from the closest prior art, the problem to be solved was to construct P_LO_L expression vectors with a better capacity at expressing cloned genes.

The solution provided was vectors wherein the cloning site for the gene to be expressed was less than 300 bp downstream from P_LO_L.

As document (31) (page 74) taught away from constructing such vectors in order to solve the above stated problem, the Appellants had gone against a prejudice, which was a clear indication of inventive step.

The technical contribution to the art was, thus, the selection of the specific position where to put the gene to be expressed to obtain an improved level of expression compared to the levels already achieved in the closest prior art. Evidence was provided by the Respondents themselves in document (A3) that the level

of trpA gene expression was enhanced when using vectors carrying as the trpA gene 115bp or 321bp downstream from P_LO_L compared to that obtained when using vectors carrying the trpA gene further downstream from P_LO_L (document(31)). The same conclusion could be arrived at from document (78).

Documents (45) and (46) showed that the yields of proteins obtained from a recombinant vector such as claimed were very high. The relatively unimproved level of expression obtained in document (72) was due to the structural properties of the specific gene which was then expressed. It represented an isolated case.

Auxiliary request
Article 123(2) EPC

A basis for the subject-matter of claim 1 was found on page 6, lines 23 to 26 of the application as filed. The skilled person would understand this passage as implicitly disclosing a vector with only 150 bp of λ DNA between P_LO_L and the N coding region.

VIII. The Appellants requested that the decision under appeal be set aside and that the patent be maintained on the basis of claims 1 to 9 for all Contracting States except AT and on the basis of claims 1 to 7 for Contracting State AT, filed on 11 May 2001 (main request) or on the basis of claims 1 to 8 filed during the oral proceedings (auxiliary request).

The Respondents requested that the appeal be dismissed.

Reasons for the Decision

Article 114(2) EPC: admissibility of the new main request

1. Claim 1 corresponds to granted claim 6 with the amendment that the reference to prokaryotic DNA sequences was omitted from the claim. This amendment was carried out to take into account the objections raised in the Board's communication. It is a well-established practice of the EPO Boards of Appeal that amendments may be accepted when filed in response to observations made by the Board (see page 507 of "Case Law of the Boards' of Appeal of the European Patent Office, 3rd Edition 1998). In accordance with this practice, the new main request is accepted into the proceedings.

Main request
Article 84 EPC

2. Part (c) of claim 1 reads: "*a DNA sequence coding for a eukaryotic or viral protein, polypeptide, enzyme, hormone, antigen or fragment thereof...*" In the Board's judgment, this wording, when read with reasonable technical skill, has to be understood such that the qualifying features "eukaryotic" and "viral" apply not only to the contiguous word "protein" but also to the biochemical entities which follow. It would be well within the ability of the skilled person to realise that hormones are eukaryotic proteins. Moreover, the argument that prokaryotic polypeptides or enzymes could still be considered as comprised within the claim because they were mentioned expressis verbis in the corresponding granted claim 6 is not convincing because claim 1 is to be read as it is and, then, it does not include this possibility. The wording of the claim is clear.

3. An objection under Article 84 EPC was also raised by the Respondents for **lack of support** in the description for the subject-matter of claim 1 which was identically that of granted claim 6. This objection will not be taken into consideration as the only objections which may be raised under Article 84 EPC in opposition proceedings are those relating to amendments which were carried out in the course of said proceedings (eg. T 301/87, OJ 1990, 335).

4. The main request is allowable under Article 84 EPC.

Article 56 EPC, claim 1

5. Document (31) was identified by both parties as the closest prior art although document (30) was also cited by the Respondents in this respect. Since document (31) provides a short discussion of the potential developments to be foreseen for $\lambda P_L O_L$ vectors (page 74) as well as the same informations as document (30), it is the more relevant document of the two, ie the closest prior art for the purpose of assessing inventive step.

6. Document (31) discloses plasmid cloning vehicles which promote gene expression from $P_L O_L$, in particular pHB2. pHB2 contains neither the <u>cro</u> gene nor the <u>N</u> gene in active form, and carries four cloning sites for insertion of the gene to be expressed: EcoRI, BamHI and SalI are situated at least 600 bp downstream from $P_L O_L$ (page 68), whereas HpaI is situated about 300 bp downstream from the promoter region. On page 74, it is stated : " *It is reasonable to assume that genes inserted into the HpaI restriction site upstream from the three sites used in this study will be transcribed with similar efficiency*".

7. Starting from the document (31), the problem to be solved may be defined as the provision of plasmid cloning vectors which lead to improved gene expression.

8. The proposed solution is plasmid vectors wherein the cro and N genes are inactive, gene expression is regulated from P_LO_L and the cloning site for the gene to be expressed is situated less than 300 bp downstream from the regulatory region.

9. The Appellants argued that the statement on page 74 of document (31) (see point 6 above) taught the skilled person away from cloning the gene to be expressed less than 300bp downstream from P_LO_L in order to solve the above mentioned problem and that, therefore, the invention was carried out inspite of a prejudice in the art. The Board is not convinced that this is the case: this statement is worded by its authors as a mere assumption and is not supported by any scientific explanation. Thus, the skilled person would consider it as merely speculative and would not refrain from using cloning sites less than 300bp downstream from P_LO_L on its account. Furthermore, it does not constitute a prejudice within the meaning given to the word in the case law of the Boards of Appeal, ie an opinion or preconceived idea widely or universally held by experts, the overcoming of which may justify acknowledging inventive step (cf. decisions T 631/89 of 14 April 1992 and T 695/90 of 31 March 1992).

10. The claimed plasmids would only be a solution to the given problem if an enhanced level of expression is indeed achieved. This could be demonstrated by comparing sets of data in which gene expression is carried out under the same conditions from vectors having inserted the gene to be expressed less than

300 bp downstream and more than 300 bp downstream from P_LO_L, respectively. The documents cited in this context will be reviewed in the points 11 to 14 below.

11. Document (A3) (pages 2 and 8) shows that the level of expression of the S.dysenteriae trpA gene is about the same irrespective of whether said gene is inserted in the expression vectors some 115bp or 321bp downstream from P_LO_L as strains transformed by said vectors express the trpA protein in a proportion of from about 3% to about 5% relative to the total protein content, as measured by the method described in the patent in suit (SDS polyacrylamide gel of newly synthesized radioactive proteins). As such, this result does not demonstrate that an enhanced expression is obtained by approaching the gene to be expressed from the regulatory region. The Appellants argued that the observed level of expression is an improvement compared to the level of expression of the trpA gene inserted 600 bp downstream from P_LO_L (document (31), page 69) as the amount of trpA protein protein in the latter case corresponds to 2% of the total protein content of the cells, as inferred from the enzyme activity. In the Board's judgment, as the means of measuring the amount of trpA protein is different from that used in document (A3), it is not possible to draw any meaningful conclusion from comparing these results.

12. Document (78) is a letter from a scientist to his colleague where he recalls having compared the level of trpA enzyme obtained from strains transformed by plasmids containing the trpA gene inserted 600bp (pHUB2trpA) and 115bp downstream from P_LO_L (pSRK2311A1), respectively. It is stated: " *Unfortunately, I learned during the experiment that my trpB-protein-prep., which is needed to determine trpA, had decreased in its activity and that limited my supply. I could therefore perform only few tests bringing the range of*

experimental error pretty high, possibly 30%. Nevertheless, the results show that pSRK2311A1 did well in the experiment and gave approximately 6% of total cell protein as trpA, pHUB2trpA gave 2.7%". The Board is not convinced that a conclusive weight may be given to these results in view of the limited number of tests which are said to have been carried out, resulting in a high experimental error which would render the results unconvincing in view of the question to be answered here, ie whether the claimed vector reliably provides enhanced expression.

13. The Appellants pointed to documents (45) and (46) as evidence that high levels of prokaryotic and viral proteins were obtained by using vectors such as claimed. These documents, however, do not disclose comparative data and it is not possible to conclude from them that in the experimental conditions which they describe, the same high levels of expression would not have been obtained by using a plasmid where the gene to be expressed would be inserted further downstream from P_LO_L. Rather, at face value, the high level of expression observed might be due to the use of the P_LO_L regulatory region as such and not to its position relative to the site of insertion of the gene.

14. Finally, there is document (72) which discloses plasmids for high level expression of diphteria toxin peptides. The most efficient of these plasmids is a plasmid which comprises on a 356 bp DNA fragment, P_LO_L and the N gene Shine-Dalgarno sequence (page 1010, right hand column). It is said on page 1008 that it is the presence of the Shine-Dalgarno sequence which is responsible for the increased level of peptide production. It would thus appear that other features than those of the claimed vector are decisive for achieving an enhanced level of expression.

15. It is, thus, concluded that there is no reliable side by side comparison data to support the hypothesis that the plasmids of the invention are better than that of the prior art. The technical effect which gives rise to the problem to be solved (enhanced expression) has not been demonstrated to be achieved by the technical features of the claimed invention (features a) to c)). Thus, the question of whether an inventive link would exist between said technical features and said technical effect does not arise.

16. The reasons for lack of inventive step developed in points 11 to 15 above are based on the problem defined in point 7. Had the problem been seen as providing an alternative plasmid to that described in document (31), in accordance with the case law of the Boards of appeal, the achievement of a surprising effect is no precondition for the existence of inventive step (cf. T 154/87 of 29 June 1989). All that is necessary is that the claimed subject-matter could not be derived by the skilled person in an obvious manner from the prior art (cf. T 426/92 of 3 March 1994). Document (31) in its introduction emphasizes the need for constructing efficient vectors and describes the features of such vectors as being, in particular, a strong promoter under the control of a regulatory gene and unique restriction sites for insertion of DNA downstream from the promoter. On page 61, it is also suggested that the gene to be expressed be inserted at several of the unique restriction sites. The claimed vector is a specific example of the vectors, the isolation of which is proposed in document (31) in that the cloning site is situated less than 300bp downstream from the promoter. Yet, at the priority date, the sequence of the λ DNA downstream from P_LO_L was known from document (34). It was thus obvious to identify the unique restriction sites in this DNA and to use them as cloning sites for the genes to be expressed.

17. For these reasons, the main request fails to fulfil the requirements of Article 56 EPC.

Auxiliary request
Article 123(2) EPC; claim 1

18. In claim 1 of this request, the positioning of P_LO_L in the vector is defined not only by its distance to the cloning sites but also by its distance to the beginning of the N gene. The Appellants point out to the passage on page 6, lines 23 to 26 of the application as filed: "*Preferably the distance between the chosen promoter and the recognition sites is ...less than about 150 base pairs*" as a basis for these two features, arguing that it would be implicit for the skilled person that if the cloning site is located less than about 150bp downstream from P_LO_L, then the beginning of the N gene would also have to be at this distance.

19. The Board notices that the application as filed (page 19) provides constructs in which the cloning site is located less than 300 bp downstream from P_LO_L as well as being within the N gene. For such constructs, it is implicit that the distance between P_LO_L and the beginning of the N gene is less than 300bp. Yet, the application as filed is wholly silent as to the structure of constructs where the distance between P_LO_L and the cloning site is less than 150bp. In particular, the "less than about 150bp DNA" need not be λ DNA nor have the cloning sites to be those included in the N gene. Thus, in claim 1, the term "less than 150bp" is given a new interpretation which is not disclosed either explicitly or implicitly in the application as filed. Accordingly, claim 1 does not fulfil the requirements of Article 123(2) EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar The Chairwoman

P. Cremona U. Kinkeldey