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Datasheet for the decision of 1 February 2007

Case Number: T 0905/05 - 3.3.08

Application Number: 92901454.6

Publication Number: 0560885

IPC: C12N 15/31

Language of the proceedings: EN

Title of invention:
Recombinant cells that highly express chromosomally-integrated heterologous genes

Patentee:
University of Florida

Opponents:
(1) NOVOZYMES A/S
(2) Koninklijke DSM N.V.
(3) GENENCOR INTERNATIONAL INC.

Headword:
Gene expression/U. OF FLORIDA

Relevant legal provisions:
EPC Art. 56, 83

Keyword:
"Main request - inventive step - yes"
"Sufficiency of disclosure - yes"

Decisions cited:
G 0001/93, G 0004/93, T 0256/87, T 0606/89, T 0019/90, T 0455/91, T 0694/92, T 0860/93

Catchword:
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Case Number: T 0905/05 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 1 February 2007

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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 12 May 2005 concerning maintenance of European patent No. 0560885 in amended form.

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
C. Rennie-Smith

Summary of Facts and Submissions

I. European patent No. 0 560 885 with the title "Recombinant cells that highly express chromosomally-integrated heterologous genes." was granted with 41 claims for all Designated Contracting States, based on the International patent application No. WO 92/10561.

Granted claim 1 read as follows:

"1. A process for producing a recombinant host cell that produces high levels of a desired polypeptide comprising the steps of:

(a) transforming one or more host cells with a nucleic acid molecule comprising

(i) a heterologous polynucleotide segment comprising a sequence encoding a desired polypeptide, and

(ii) sequences that flank said heterologous polynucleotide segment and are homologous to a host gene under transcriptional control of an endogenous promoter,

whereby chromosomal integration into said host gene of said heterologous polynucleotide segment results by means of homologous recombination;
(b) selecting for one or more host cells produced in step (a) that express the polypeptide;
(c) exposing one or more host cells identified in step (b) to a mutagen under conditions such that a mutation that causes increased expression of said heterologous polynucleotide segment is created in said chromosome; and then

(d) testing host cells produced in step (c) for host cells that produce said desired protein at a level higher than said initial level, to obtain host cells having a mutation that causes increased expression of said heterologous polynucleotide segment resulting in an increase in production by said host cells of said desired polypeptide compared to said production of said desired polypeptide by said host cells in the absence of said mutation, wherein said increased expression is retained in the absence of conditions that select for cells having said increased expression."

Dependent claims 2 to 25 related to further features of the process of claim 1. Claims 26 and 27 were directed to recombinant host cells obtainable by the process of claim 1. Claims 28 to 35 related to further features of the recombinant host cells of claims 26 or 27.
Claims 36 to 41 related to specific recombinant E.coli strains defined by their deposit ATCC numbers.

II. Three oppositions were filed under Article 100(a) to (c) EPC. The opposition division maintained the patent in amended form pursuant to Article (106(3)) EPC on the basis of the second auxiliary request then on file comprising claims 1 to 6 corresponding to granted claims 36 to 41. The main request, namely the granted claims, was refused for lack of novelty; the first auxiliary request then on file was refused for lack of inventive step.

III. The appellant (patentee) filed a notice of appeal, paid the appeal fee and submitted a statement of grounds of appeal together with the same main request as was refused by the opposition division (granted claims)and

eight auxiliary requests, the last one of them being the claims as maintained by the opposition division.

IV. Respondents I to III (opponents 1 to 3) filed submissions in answer to the statement of grounds of appeal.

V. The board sent a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal indicating its preliminary, non-binding opinion.

VI. The appellant and respondent II filed further submissions in answer to this communication. The appellant's submissions were accompanied by three further auxiliary requests to be considered as auxiliary requests VII to IX, earlier requests VII and VIII being renumbered X and XI.

VII. Oral proceedings took place on 1 February 2007. The appellant replaced all requests on file by a main request comprising 29 claims.

Claim 1 read as follows:

"1. A process for producing a recombinant **bacterial** host cell that produces high levels of a desired polypeptide comprising the steps of:

(a) transforming one or more host cells with a nucleic acid molecule comprising

(i) a heterologous polynucleotide segment comprising a sequence encoding a desired polypeptide, and

(ii) sequences that flank said heterologous polynucleotide segment and are homologous to a host gene under transcriptional control of an endogenous promoter,

whereby chromosomal integration into said host gene of said heterologous polynucleotide segment results by means of homologous recombination **and said heterologous polynucleotide segment is under the transcriptional control of the endogenous promoter by virtue of being integrated into a host cell chromosome on the downstream side of the promoter**;

(b) selecting for one or more host cells produced in step (a) that express the polypeptide;

(c) exposing one or more host cells identified in step (b) to a mutagen under conditions such that a mutation that causes increased expression of said heterologous polynucleotide segment is created in said chromosome; and then

(d) testing host cells produced in step (c) for host cells that produce said desired protein at a level higher than said initial level, to obtain host cells having a mutation that causes increased expression of said heterologous polynucleotide segment resulting in an increase in production by said host cells of said desired polypeptide compared to said production of said desired polypeptide by said host cells in the absence of said mutation, wherein said increased expression is retained in the absence of conditions that select for cells having said increased expression." (differences to granted claim 1 are indicated by emphasis added by the board).

Dependent process claims 2 to 23 corresponded to granted claims 2 to 10, 13 to 25. Claims 24 to 29 which related to specific recombinant E.coli strains defined by their deposit ATCC numbers were the claims accepted by the opposition division.

VIII. The following documents are mentioned in this decision:

(1): EP-A-0 284 126;

(3): Makino, O. et al., Agric.Biol.Chem. Vol.50, No.2, pages 501 to 504, 1986;

(17): Sakai, A. et al., Genetics, Vol. 119, pages 499 to 506, July 1988;

(23): Cregg, J.M. et al., Genetics and Molecular Biology of Industrial Organisms, Am.Soc.Microbiology, Washington, Editors: Hershberger et al., pages 343 to 352, 1989.

IX. The appellant's submissions in writing and during oral proceedings insofar as relevant to the present decision may be summarised as follows:

Article 123(2) EPC; added subject-matter

The application as filed disclosed bacterial host cells eg. on page 9, line 11, page 16, line 2, page 18, line 16, page 35, line 12. The expression "encodes a plurality of polypeptides" found a basis on page 6, lines 32 or 36, page 7, line 8 and page 14, line 31. The requirements of Article 123(2) EPC were fulfilled.

Article 84 EPC; clarity

It was unambiguous that in the expression "by virtue of being integrated into a host cell chromosome on the downstream side of the promoter", the term "on the downstream side of the promoter" meant "downstream from and outside of" the promoter as this was the intended meaning throughout the patent specification eg. page 8, lines 17 to 34, page 14, lines 20 to 25, page 15, lines 1 to 4 of the application as filed.

Article 56 EPC; inventive step

- At the priority date, the skilled person already had many techniques at his/her disposal to increase the production of a polypeptide. In bacteria, a preponderant method was to express the relevant gene from multicopy plasmids. Yet, it was also known to increase transcription by using strong promoters, to adapt codon usage to the host cell, to improve secretory capacities, to mutagenize and select mutants with a higher expression level of the relevant polypeptide. The patentee had chosen a distinctively different way when devising a method which combined homologous recombination and mutagenesis and this method had multiple advantages.

- Document (3) was the closest prior art which disclosed a method for high level expression of a desired gene which involved homologous recombination of said gene into the E.coli chromosome downstream of a strong inducible promoter. When the patentee used an equivalent method (examples of the patent in suit), a very low level of expression was obtained. Nonetheless,

it pursued the experiment by mutagenizing the recombinant E.coli strain and, then, surprisingly obtained levels of expression as good as those obtained when using multicopy plasmids. The method was, thus, clearly inventive.

Opponents' arguments that the claimed method was not inventive over the combined teachings of documents (3) and (23) were not convincing because these teachings were too far apart to be combined, document (23) being a review article on gene expression in the yeast P. pastoris.

- Document (17) was a research article about hypersecretion in S.cerevisiae. There was no reason why a skilled person would take this document into consideration when attempting to increase polypeptide production in E.coli. A fortiori, there were no reasons to combine the teachings of documents (17) and (1) when assessing inventive step.

- Finally, the opponents argued that the patent in suit did not provide any evidence that the method of claim 1 was a solution to the problem of overexpressing a desired polypeptide. Yet, at the same time, they objected that the subject-matter of this claim was an obvious solution to this problem. These two arguments were mutually exclusive. In fact, homologous recombination and mutagenesis were techniques routinely employed by the skilled person, but their hitherto undisclosed combination resulted in an unexpected level of expression and this was why the claimed subject-matter was inventive.

Article 83 EPC; sufficiency of disclosure

- The patent in suit provided detailed examples of how to integrate a heterologous polynucleotide segment - comprising Z.mobilis genes - on the downstream side of a bacterial endogenous promoter - the pfl promoter - and the resulting recombinant host cell - E.coli - expressed high levels of the two Z.mobilis proteins. The pfl promoter itself was not present in the heterologous polynucleotide segment and, thus, integration into the bacterial chromosome occurred downstream from the endogenous promoter. The skilled person would have no problems in reproducing this teaching.

- The claim was broad but, in accordance with the case law (T 19/90, OJ EPO 1990, 476), that was not in itself a reason to reject the patent for lack of sufficient disclosure. In fact, the two techniques which were necessary to put the claimed method into practice - homologous recombination and mutagenesis - were well-known to the skilled person. The respondents' arguments that the claimed method could not be reproduced were mere assumptions which had not been substantiated by any verifiable facts. The requirements of Article 83 EPC were fulfilled.

X. The respondents' submissions in writing and during oral proceedings insofar as relevant to the present decision may be summarised as follows:

Article 123(2); added subject-matter

The terms "bacterial host cells" (claim 1) and "encoding a plurality of polypeptides" (claim 4) had no basis in the application as filed. Therefore, the main request did not comply with the requirements of Article 123(2) EPC.

Article 84; clarity

The meaning of the expression "by virtue of being integrated into a host cell chromosome on the downstream side of the promoter" was unclear in two respects. Firstly, it was doubtful whether the "promoter" was the endogenous promoter present in the bacterial chromosome. Secondly, the term "on the downstream side of the promoter" could equally mean "within the promoter, in the downstream part of it" or "outside the promoter, downstream from it".

Article 56 EPC; inventive step

- The subject-matter of claim 1 lacked inventive step over the teachings of document (3) combined with those of document (23), alternatively, over the teachings of document (17) combined with those of document (1) or with the prevailing common general knowledge at the priority date. Finally, there was also lack of inventive step as the patent in suit did not produce any evidence that the problem which the claimed subject-matter purported to solve had, in fact, been solved.

- Document (3) taught that in order to produce a polypeptide on a large scale in E.coli, it may be advantageous to insert the corresponding gene into the chromosome and express it therefrom under the control of a strong inducible promoter. The method used to obtain the recombinant host cell corresponded to the first step of the now claimed method.

The problem to be solved could, thus, be defined as providing a method to increase the level of foreign gene expression once it was inserted in the bacterial chromosome and the solution thereto was to mutagenize the recombinant clones and select for those exhibiting a high level of expression.

This solution was obvious insofar as isolating mutants was a technique to increase gene expression which was part of the common general knowledge. Evidence thereto could be found for example in document (23), page 349. Admittedly, this document related to gene expression in yeasts, yet this was not relevant as the skilled person knew that mutagenesis could be carried out on any organisms.

- Document (17) disclosed a method to increase the production of a polypeptide to be carried out in yeast cells which corresponded to the method now claimed for bacteria. The problem to be solved could, thus, be formulated as finding alternative host cells to produce high levels of a polypeptide, and the solution was to use bacterial cells instead of yeast cells. At the priority date, bacterial cells had long been known as industrial microorganisms as was reflected in document (1). Thus, they were an obvious alternative to yeast

cells. In this context, reference was made to the earlier decision T 455/91 of 20 June 1994 where it was established that a skilled person working in one area of genetic engineering would regard a means found possible in a neighbouring area of genetic engineering as being usable in his own area, if this transfer of technical knowledge appeared to be easy and involved no obvious risks. Here, it was well known that the relevant techniques which had been used in yeast were also suited for bacteria.

- Finally, account should be taken of the fact that the examples given by the patent in suit did not illustrate the claimed subject-matter. Thus, the patentee had provided no evidence that the problem had been solved.

Sufficiency of disclosure

- The patent in suit failed to provide a teaching reproducible over the scope of the claim which covered producing recombinant bacterial host cells irrespective of the kind of bacteria by transforming them with any gene or combination thereof and, furthermore, which involved any kind of mutagenesis, even relying on natural chance events. On the contrary, there was only one example provided of the overproduction by E.coli of gene products involved in ethanol production. The situation was essentially the same as that encountered in case T 694/92 (OJ EPO 1997,408) where sufficiency of disclosure had been denied.

- The patent in suit failed to provide any information on how to ensure that chromosomal integration by homologous recombination would occur on the downstream

side of the promoter and result in expression of the gene encoding the desired polypeptide from the endogenous promoter. One could conceive of an integrative event involving homologous recombination, eg. those occurring in the flanking sequence downstream from the gene to be expressed at high level, which would result in the expression of the desired polypeptide, yet not from the endogenous promoter, and this integrative event would be indistinguishable from a homologous recombination such as claimed. In accordance with the case law (T 256/87 of 26 July 1988), in such a situation, the requirement of sufficiency of disclosure was not fulfilled.

XI. The appellant requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request filed during the oral proceedings.

The respondents requested that the appeal be dismissed.

Reasons for the decision:

Article 123(2) EPC; added subject-matter

1. Respondent II' s arguments as regards added subject-matter was that the two expressions "bacterial host cell" (claim 1) and "encoding a plurality of polypeptides" (claim 4) could not be found in the application as filed.

2. The purpose of Article 123(2) EPC is explained in the Enlarged Board's decision G 1/93 (OJ EPO 1994, 541, point 9 of the decision):

"With regard to Article 123(2) EPC, the underlying idea is clearly that an applicant shall not be allowed to improve his position by adding subject-matter not disclosed in the application as filed which would give him an unwarranted advantage and could be damaging to the legal security of third parties relying on the content of the original application."

3. Applying Article 123(2) EPC is, thus, not a question of whether or not the same words are present in a claim and in the application as filed but whether the claimed technical teaching ("subject-matter") is clearly and unambiguously derivable from that application.

4. That the now claimed process is to be performed with recombinant bacterial host cells is, in fact, the gist of the invention as described in the application as filed, starting on page 1, lines 19 to 24 going to eg, the passage bridging page 8, line 14 to page 9, line 13, page 16, lines 2 to 8, page 18, lines 6 to 26, etc.... Furthermore, the examples describe the isolation of recombinant E.coli bacterial host cells overproducing Z.mobilis gene products involved in the production of ethanol.

5. In the same manner, the fact that bacterial host cells may be transformed by a heterologous polynucleotide segment encoding a plurality of polypeptides is straightforwardly derivable from the technical teaching in the application as filed that the segment may comprise a plurality of genes, eg. on page 6, lines 30 to 32:

"*The present invention pertains to recombinant host cells that express chromosomally-integrated heterologous genes encoding useful polypeptides at high levels.*"

6. For these reasons, the requirements of Article 123(2) EPC are fulfilled.

Article 84 EPC; clarity

7. Respondent I argued that, in claim 1, the wording "and said heterologous polynucleotide segment is under the transcriptional control of the endogenous promoter by virtue of being integrated into a host cell chromosome on the downstream side of the promoter" left some doubts as to which promoter was being referred to in the second half of the sentence. For the board, it is unambiguous that the "promoter" must be the endogenous promoter referred to in the first half of the sentence, it being in any case the only promoter mentioned in the claim.

8. Respondent II argued that in that same passage the wording *"on the downstream side of the promoter"* could mean "outside of the promoter, downstream from it" or "within the promoter, in the downstream part of it" and that, therefore, there was uncertainty as to which integration event the claimed process would involve. In support of this argument, he observed that the polynucleotide segment which was made use of in the examples comprised in the upstream flanking sequence 140 base pairs containing a promoter sequence where homologous recombination could take place as well as in the rest of said sequence.

9. In accordance with the case law (T 860/93, OJ EPO 1995, 47), the description may be used to interpret the claims. Here the description in its generic part makes a clear and consistent distinction between the promoter and the gene which "follows" it. It teaches that the heterologous DNA segment should be integrated within the gene (eg. page 8, lines 17 to 24, page 14, lines 20 to 25). As for the above mentioned examples, they are wholly silent as to the presence of a promoter in the 140 base pairs fragment. Thus, irrespective of whether or not an active promoter is indeed found in this fragment, the skilled person is not made aware of it by reading the description. Accordingly, the board concludes that on the basis of the technical disclosure provided, he/she would understand the expression "on the downstream side of" as meaning "outside of the promoter, downstream from it".

10. The requirements of Article 84 EPC are fulfilled.

Article 54 EPC; novelty

11. Lack of novelty was the reason for refusal of the then pending main request (granted claims, see I above) by the opposition division. Claim 1, which was directed to a process for producing a recombinant host cell that produced high levels of a desired polypeptide, was found to lack novelty over the teachings of document (17) which related to S.cerevisiae. Present claim 1 (and dependent claims thereof) is limited to a process to be carried out in bacteria (see section VII supra). Document (17) is no longer relevant to novelty and as there are no other prior art documents on file

disclosing a process such as that now claimed, the requirements of Article 54 EPC are fulfilled.

Article 56 EPC; inventive step

12. Documents (3) or (17) were referred to as the closest prior art. The former is concerned with large scale production of a polypeptide in E.coli, the latter deals with the isolation and characterisation of mutants which show an oversecretion phenotype in S.cerevisiae.

13. In accordance with the case law (T 606/89 of 18 September 1990), the closest prior art for assessing inventive step is normally a prior art document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common.

14. The purpose of the present invention is to produce high levels of a desired polypeptide in bacteria. Therefore, the closest prior art is document (3). This document teaches that overproduction of a desired peptide is most often achieved by expressing the corresponding gene in host cells from multicopy plasmids. The poor reproducibility of the system is pointed out (page 501, left-hand column) and the authors suggest a different method involving the integration of the gene of interest into the chromosome downstream from a strong inducible promoter. The general procedure for integration, which is mentioned in the paragraph bridging pages 503 and 504, requires that the gene of interest be flanked by sequences homologous to the host gene which is naturally expressed from a strong

promoter. After transformation of the host cells, the integration at the gene locus on the chromosome is said to occur by homologous recombination downstream from the ribosome-binding sequence, namely downstream from the strong promoter. The results obtained with the recA gene as a model case for large scale production of a polypeptide (recA protein) show that, while somewhat lower than that obtained by expression from multicopy plasmids, the yield of recA protein obtained by expression from the chromosome is much more reproducible (passage bridging pages 501 and 502). There is no hint in document (3) that the method therein described is in need of any improvement.

15. Starting from the closest prior art, the problem to be solved may be defined as providing another method for producing a polypeptide at high levels in bacterial cells.

16. The solution provided is a method in which the step of integration into the chromosome by means of homologous recombination on the downstream side of an endogenous promoter is followed by a mutagenesis step coupled to the selection of highly producing clones.

17. In the absence of any suggestion in document (3) that the method proposed therein is not fully satisfactory, the skilled person wanting to solve the above mentioned problem would most probably follow the provided teaching that strong promoters were the answer. Thus, the solution immediately coming to mind would be to try and isolate constructs which would be expected to integrate downstream from any other E.coli promoters

known to be strong. Unless associated with unexpected effects, this course of action would be obvious.

18. However, that was not the solution chosen by the appellant. Instead, a step of mutagenesis was added and a high level of expression was achieved. At the priority date, mutagenesis was only one amongst the many methods which had already been tried for altering the expression level of a bacterial gene. As already mentioned, one of the "preferred ones" was using multicopy plasmids, but one could also try to achieve gene duplication in the chromosome (document (1)), to adapt codon usage etc... Thus, in the board's judgment, the present method which, in fact, consists of the combination of, firstly, putting oneself in the unfavourable position of expressing only one copy of the gene - albeit possibly strongly - and, secondly, "correcting" the level of expression obtained by altering the genetic background of the host cells rather than manipulating the gene of interest, was unexpected.

19. In this context, reference was made to document (23) as evidence that mutagenesis was a favoured approach for improving gene expression (page 349, left-hand column). However, this document is a review of the expression of foreign genes in the methylotrophic yeast Pichia pastoris. For the board, such a document could only be found and its teachings combined with those of document (3) with hindsight knowledge of the invention. And, besides, as already mentioned, inventive step does not reside in any one of the steps of the claimed method taken separately but in their combination.

20. The claimed subject-matter was also argued to be obvious in the light of the teachings of document (17) combined with those of document (1). Document (17) is a research article on the possible genetic determinants of the oversecretion phenotype in S.cerevisiae. Two kinds of mutants are isolated which oversecrete a protein encoded by a foreign gene integrated by homologous recombination into the yeast chromosome downstream from a strong promoter. The purpose of the study is to investigate their genetic identity: one of them is found to be altered in the secretory pathway whereas the other exhibits an increased transcription level.

21. In the board's judgment, the skilled person would have no reason to consider document (17) when wanting to achieve overproduction of a protein in a bacterial host. And the information in document (1) - which was relied upon by respondent I in combination with document (17) - that bacteria are industrial microorganisms does not change anything in this respect.

22. In this context, reference was also made to the earlier decision T 455/91 (supra). In this case, vectors suitable for expressing any exogenous gene in yeast cells were claimed, which comprised genetic elements corresponding to those of vectors as were known in the bacterial art for the same purpose. The then competent board concluded that a skilled person working in one area of genetic engineering would regard a means found possible in a neighbouring area of genetic engineering as being useable in his own area, if such a transfer of technical knowledge appeared to be easy and to involve no obvious risks and, on that basis, inventive step was

denied. That situation, however, is quite different from the present insofar as the then relevant bacterial art did address the same problem as the then patent in suit and, thus, "came to mind" when trying to solve the technical problem. As this is not presently the case - see paragraph 20 supra-, the findings of T 455/91 (supra) do not apply.

23. Finally, the argument was presented that the patent in suit did not provide any evidence that the problem mentioned in point 15, supra had been solved because the examples given were not suitable to illustrate the claimed solution. There existed the possibility that in the heterologous polynucleotide segment which was exemplified, homologous recombination took place within the 140bp part of the flanking sequence which contained a promoter. In other words, the gene of interest would not necessarily be under the transcriptional control of the endogenous promoter by virtue of being integrated on the downstream side from the promoter. However, it was not denied that the relevant flanking sequence contained much more DNA than the 140bp fragment nor that the rest of this DNA was homologous to the gene sequence downstream from the promoter on the chromosome and, thus, may equally participate in homologous recombination. Accordingly, and in the absence of any evidence that homologous recombination would only take place in the 140 base pairs fragment, it must be concluded that the argument is not adequately substantiated.

24. For these reasons, inventive step is acknowledged.

Article 83 EPC; sufficiency of disclosure

25. At oral proceedings, the fact that the techniques needed to put the claimed method into practice, namely homologous recombination and mutagenesis, were routine techniques in the bacterial field was not challenged.

26. The first argument which was raised against sufficiency of disclosure was that the scope of claim 1 was much too wide and, thus, unwarranted, taking into account the scant technical teaching provided. In this context, the findings in the earlier decision T 19/90 (supra, point 3.3 of the decision) are particularly relevant:

"*However, the mere fact that a claim is broad is not in itself a ground for considering the application as not complying with the requirement for sufficient disclosure under Article 83 EPC. Only if there are serious doubts, substantiated by verifiable facts, may an application be objected to for lack of sufficient disclosure.*"

27. Although it was suggested that it would be inevitable that the invention could not be reproduced in at least some embodiments, no serious doubts were raised and no verifiable facts were produced, and , accordingly, the argument is not found convincing.

28. Decision T 694/92 (supra) was also referred to, wherein sufficiency of disclosure was denied to a method for genetically modifying a plant cell by transferring into it a T-DNA comprising a plant promoter and a plant structural gene so that the protein encoded by the plant gene would be expressed. In this earlier case, it

was known from the art that no previous attempts to transfer genes into plant cells had resulted in the expression of these genes. The method itself involved a new approach and the expression of only one plant gene - encoding phaseolin - was observed but at barely detectable levels. The then competent board reasoned that the technical teaching provided was not sufficient for the skilled person to reliably achieve without undue burden the technical effect of expression in any plant cell of any plant gene under the control of any promoter. The facts of that case are, however, clearly not comparable to those of the present case and, thus, the conclusion also has no bearing on the present case.

29. Yet another argument was that the skilled person was given no technical means to find whether or not he/she was reproducing the invention, a situation which, in accordance with the case law (T 256/87, supra) amounted to the requirement of Article 83 EPC not being fulfilled. This argument was illustrated at oral proceedings by a diagram depicting what would happen if integration occurred into the chromosome by means of one cross-over with the flanking sequence situated downstream from the gene of interest in the heterologous polynucleotide segment - ie. the sequence carrying the transcription terminator. From this diagram, it was deduced that once integrated into the chromosome, the gene of interest would not be expressed from the endogenous promoter yet would nonetheless be expressed. In the respondent's opinion, this implied that the depicted event could not be distinguished from that which the claimed process involved.

30. In the board's judgment, sketching a possible recombination event on a flipchart, without providing any experimental evidence that if it occurred in vivo and was followed by a step of mutagenesis, it would result in bacterial cells such as obtained by the claimed process - producing high levels of a desired polypeptide in a stable manner- illustrates no more than an assumption that it would. Mere assumptions are not adequate evidence for a conclusion of lack of sufficient disclosure. Accordingly, the findings in decision T 256/87 (supra) are not relevant to the present case.

31. For these reasons, and in the absence of any evidence to the contrary, sufficiency of disclosure is acknowledged.

Further matter:

32. In addition to the method claim 1 and its dependent claims 2 to 23, the claim request comprises claims 24 to 29 which relate to specific, deposited recombinant E.coli host cells as defined by ATCC deposit numbers. These six claims are those which were accepted by the opposition division. The respondents did not appeal against the decision of the opposition division. Therefore, these claims are not for consideration by the board (Enlarged Board of Appeal decision G 4/93 OJ EPO 1994, 875).

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 the main request filed during the oral proceedings and a description and drawings to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski L. Galligani