Decision
of 29 January 2003

Case Number: T 0437/98 - 3.3.8
Application Number: 91901587.5
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Language of the proceedings: EN

Title of invention: STABLE INTEGRATION OF DNA IN BACTERIAL GENOMES

Patentee: Novozymes A/S

Opponent: Genencor International, Inc.

Headword: DNA integration/NOVOZYMES

Relevant legal provisions:
EPC Art. 54, 56, 69(1), 84
EPC R. 67, 68(2)

Keyword:
"Main request - clarity (no)"
"First and second auxiliary requests - clarity (no)"
"Third auxiliary request - clarity (yes)"
"Novelty (yes)"
"Inventive step (yes)"
"Procedural violation (no)"
"Reimbursement of appeal fees (no)"

Decisions cited:
T 0094/82, T 0068/85, T 0190/99, T 0337/95, T 0292/90,

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DECISION
of the Technical Board of Appeal 3.3.8
of 29 January 2003

Appellant: Genencor International, Inc.
(Opponent)
925 Page Mill Road
Palo Alto
CA 94304-1013 (US)

Representative: Brasnett, Adrian
MEWBURN ELLIS
York House
23 Kingsway
London WC2B 6HP (GB)

Respondent: Novozymes A/S
(Proprietor of the patent)
Krogshoejvej 36
DK-2880 Bagsvaerd (DK)

Representative: Bassett, Richard Simon
Eric Potter Clarkson
Park View House
58 The Ropewalk
Nottingham NG1 5DD (GB)


Composition of the Board:
Chairman: F. L. Davison-Brunel
Members: P. Julia
M. B. Günzel
Summary of Facts and Submissions

I. The opponent (appellant) lodged an appeal against the interlocutory decision of the opposition division to maintain the European patent No. 0 506 780 in amended form on the basis of claims 1 to 42 of the main request then on file. All claims of this request were as granted except for independent claim 33.

Claims 1, 12, 25 and 33 as accepted by the opposition division read as follows:

"1. A method of producing a bacterial cell which in its genome carries an integrated non-replicative DNA construct comprising (1) a DNA sequence of interest, (2) a DNA sequence which is homologous with a region of the genome of the cell, and (3) an origin of replication, the DNA construct lacking a functional gene coding for a factor required to initiate replication from said origin of replication, the method comprising

(a) transforming bacterial cells with a parental plasmid vector which comprises a first origin of replication and a second origin of replication in the same orientation as the first origin of replication, which first and second origins of replication are sufficiently similar to be functional with the same replication factor(s), the first and second origins of replication dividing the vector into two parts, (i) a first part comprising the first origin of replication and one or more functional genes encoding the replication factor(s) required for plasmid replication from said first and second origin of replication, and (ii) a second part comprising the
second origin of replication, a DNA sequence of interest, and a DNA sequence which is homologous with a region of the genome of a cell intended for introduction of the vector, and 
(b) culturing the transformed cells under selective conditions, replication of the parental plasmid vector giving rise to the formation of a first progeny vector comprising the first origin of replication and one or more functional genes encoding the replication factor(s) required for plasmid replication from said first and second origin of replication, and a second progeny vector comprising the second origin of replication but lacking a functional gene encoding a replication factor, as well as comprising a DNA sequence of interest, and a DNA sequence which is homologous with a region of the genome of the cell, continued culturing of the transformed cells under selective conditions resulting in the integration of said second progeny vector into the bacterial genome by homologous recombination and loss of the first progeny vector as well as the parental vector from the cells.

"12. A method of producing a bacterial cell which in its genome carries an integrated non-replicative DNA construct comprising (1) a DNA sequence of interest, (2) a DNA sequence which is homologous with a region of the genome of the cell, and (3) an origin of replication, the DNA construct lacking a functional gene coding for a factor required to initiate replication from said origin of replication, the method comprising
(a) transforming bacterial cells with (i) a first DNA vector comprising a first origin of
replication and one or more functional genes encoding the factor(s) required for plasmid replication from said first origin of replication, and with (ii) a second DNA vector comprising a second origin of replication but lacking a functional gene encoding a factor required for plasmid replication from the second origin of replication, as well as comprising a DNA sequence of interest, and a DNA sequence which is homologous with a region of the genome of a cell, said first and second origins of replication being sufficiently similar to be functional with the same replication factor(s) so that replication of the second DNA vector from the second origin of replication is initiated by the replication factor(s) encoded by the gene(s) present on the first DNA vector, and (b) culturing the resulting cells under selective conditions leading to integration of said second DNA vector into the bacterial genome by homologous recombination and loss of the first DNA vector."

"25. A parental plasmid vector which comprises a first origin of replication and a second origin of replication in the same orientation as the first origin of replication, which first and second origins of replication are sufficiently similar to be functional with the same replication factor(s), the first and second origins of replication dividing the vector into two parts, (i) a first part comprising the first origin of replication and one or more functional genes encoding the replication factor(s) required for plasmid replication from said first and second origin of replication, and (ii) a second part comprising the second origin of replication, a DNA
sequence of interest, and a DNA sequence which is homologous with a region of the genome of a cell intended for introduction of the vector".

"33. A bacterial cell which cannot be transformed by being made competent and which in its genome carries an integrated non-replicative DNA construct comprising (1) a DNA sequence of interest, (2) a DNA sequence which is homologous with a region of the genome of the cell, and (3) an origin of replication, wherein the DNA construct has been deleted of a gene coding for a factor required to initiate replication from said origin of replication or wherein the gene encoding the replication factor has been modified so as to encode an inactive replication factor." (showing in bold the amendment introduced during opposition proceedings).

Claims 2 to 11, 13 to 24, 26 to 32 and 34 to 39 related to particular embodiments of the subject matter of claims 1, 12, 25 and 33 respectively. Independent claim 40 was directed to a process for producing a polypeptide of interest, comprising culturing a bacterial cell according to any of claims 33 to 39. Claims 41 and 42 related to particular embodiments of the process of claim 40.

II. The patent had been opposed in its entirety under Article 100(a) EPC on the grounds of lack of novelty of granted claims 33 to 42 (Article 54 EPC) and lack of inventive step of granted claims 1 to 42 (Article 56 EPC).

III. In the statement of grounds of appeal, the appellant maintained the objections raised in the opposition proceedings and further objected under Article 84 EPC
to the amendment introduced into claim 33 accepted by the opposition division. The decision under appeal was also considered to suffer from a lack of reasoning within the meaning of Rule 68(2), first sentence, EPC.

IV. The board issued a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal indicating, in particular, its preliminary, non-binding opinion that the amendment introduced into claim 33 at the opposition stage appeared not to be clear (Article 84 EPC).

V. In reply to the board's communication, the respondent (patentee) filed further written submissions and four auxiliary claim requests (AR1 to AR4). In auxiliary requests AR1 to AR3, claim 33 differed from claim 33 of the main request in that the feature "A bacterial cell which cannot be transformed by being made competent" was amended in the following manner:

AR1: "A bacterial cell which cannot be transformed by being made naturally competent..."

AR2: "A bacterial cell which has not been made by transformation of naturally competent cells..."

AR3: "A bacterial cell belonging to the genus Bacillus and which cannot be transformed by being made competent..."

VI. Oral proceedings were held on 29 January 2003.

VII. The following documents are cited in this decision:

D1: T. Seki et al., in "Genetics and Biotechnology of
Bacilli", Ganesan, A.T. and J.A. Hoch, eds.,

D2: M. Young and S.D. Ehrlich, J. Bacteriol. (May)
1989, Vol. 171(5), 2653 to 2656;

1987, Vol. 210, 116 to 121;

39 to 48;

D5: R. Villafane et al., J. Bacteriol. (Oct.) 1987,
Vol. 169(10), 4822 to 4829;

D10: M.F. Gros et al., The EMBO J. 1987, Vol. 6(12),
3863 to 3869;

D12: P.H. Pouwels et al., "Cloning Vectors, A
Laboratory Manual", Elsevier Science Publishers,
1985, pages IV-1 to IV-2;

D15: D. Dubnau in "Bacillus subtilis and other Gram-
Positive Bacteria". A.L. Sonenshein et al., eds.,
Am. Soc. Microbiol. 1993, Chapter 39, 555 to 561;

D16: S. Bron in "Molecular Biological Methods for
Bacillus". C.R. Harwood and S.M. Cutting eds.,
John Wiley & Sons 1990, Chapter 3, 98 to 100 and
132;

D17: H. Hiraoka et al., J. Ferm. and Bioeng. 1992,
Vol. 74(4), 241 to 243;

1964, Vol. 87(3), 519 to 526;


VIII. The appellant's submissions in writing and during oral proceedings may be summarized as follows:

Main and first auxiliary requests: claim 33
Article 84 EPC; clarity

- The amendment carried out in claim 33 of the main request which required that the bacterial cell "cannot be transformed by being made competent" was unclear for two reasons:

(i) The wording "competent" embraced natural and artificial competence and it was not sure which one was intended and,

(ii) Assuming that natural competence was intended, it remained that this state of competence depended on the bacterial strain as well as on the experimental conditions used for developing competence (documents D16 to D18). Thus, in cases when one failed to obtain competence, it was not possible to determine whether the reason for this failure was that the bacterial cell was not intrinsically capable of being competent or else that appropriate conditions had not yet been found.

- In the first auxiliary request, claim 33 was restricted to these bacterial cells which could
not be transformed by being made \textit{naturally} competent. This wording suffered from lack of clarity for the reasons explained in (ii) above.

Second auxiliary request; claim 33

Articles 123(2) EPC and 84 EPC; support in the application as filed, clarity

The definition of the claimed bacterial cell as that "which is not made by transformation of naturally competent cells" had no basis in the application as filed. Furthermore, it was unclear to define a product such as the bacterial cell by the way it had \textit{not} been made.

Third auxiliary request; claim 33

Article 84 EPC; clarity

The claimed bacterial cell was defined as belonging to the genus Bacillus in addition to the feature that it could not be transformed by being made competent. The fact that the bacterial cell would be of Bacillus origin did not change the unclarity attached to the notion of non-competence. Document D18 showed that some strains of B. licheniformis could be competent and others not, under the same conditions.

Article 56 EPC, inventive step

Claim 12

Document D2 showed that the concomitant presence in a cell, of an integrated DNA with an origin of replication and of a trans-acting replication factor recognizing said origin of replication resulted in the
instability of the integrated DNA surrounding the origin of replication, whereas the removal of the trans-acting replication factor reduced this instability.

Knowing from document D2 that it was desirable to integrate a plasmid without a functional replication gene into the genome of a cell to achieve stability of the integrated DNA, the problem to be solved could be defined as how to replicate such plasmid into the cell once it had entered and before integration in the bacterial genome.

Document D1 showed that a functional replication factor of a first plasmid could act in trans to replicate a second plasmid under conditions where this latter plasmid could not use its own temperature sensitive replication factor.

There was no inventive skill in the method of claim 12 because this method only combined the step of using the "double vector system" of document D1 to maintain into the cell the non-replicative vector containing the DNA of interest with the further step of removing the trans-acting replication factor as taught in document D2 in order for the DNA sequence of interest to integrate into the genome in a stable manner.

Claims 1 and 25

The difference between the method of claim 1 and that of claim 12 was that a single plasmid ie the parental plasmid vector with two origins of replication was used in the earlier instead of two plasmid vectors in the latter. Yet, the parental plasmid vector divided into...
the two separate plasmids of claim 12 following transformation. Claim 25 related to the parental plasmid vector _per se_.

Document D10 taught that a plasmid of the same class as the parental plasmid vector replicated using rolling circle replication and that, if it had two origins of replication, recombination would occur between the origins and provide daughter plasmids, each containing one part of the parental plasmid. The skilled person would have no technical prejudice against providing the two plasmids used in the method of claim 12 as a single plasmid. Accordingly, the feature of one plasmid having two origins of replication did not change the conclusion of lack of inventive step reached in relation to claim 12.

**Claim 33**

The methods of claims 12 or 1 were not necessary to isolate the bacterial cell of claim 33.

The starting point for the assessment of the inventive step of the bacterial cells of claim 33 was document D2 which, as already mentioned, disclosed that the DNA of interest could be stably maintained in the genome of bacterial cells in the absence of DNA replication. Document D4 taught that the in situ DNA replication could be regulated by using a plasmid thermosensitive for replication.

Thus, the skilled person would find it obvious to obtain the claimed bacteria by artificially transforming the non-competent starting cells with a vector containing a thermosensitive origin of
replication, replicating said vector at the permissive temperature in order to allow the integration of the DNA of interest into the bacterial genome, and culturing at the non-permissive temperature, thus obtaining bacterial cells with a stable integrated, non-replicative vector containing the DNA of interest. Nothing inventive could be seen in the selection of non-competent cells as document D12 already showed that vector systems used for competent B. subtilis could be used for general Bacilli, including non-competent ones.

**Rule 68 EPC; procedural violation**

In section 4.2 of their decision, the opposition division had stated that "no objections have been raised by the Opponent against claims 33-39 (main request)....". This statement was wrong. By ignoring the fact that the opponent did have objections to said claims, the opposition division failed to take them into account.

Besides, insofar as claims 33 to 39 (but not the appellant's arguments) were considered by the opposition division, they were only considered in relation to novelty. As regards the opposition division's reasoning on inventive step, the same statement that the "prior art was absolutely silent with regard to the stable integration of non-replicative DNA in the bacterial genome by transformation of bacterial cells which cannot be transformed by being made competent", which was essentially a statement relevant to novelty had only been repeated.

Both these actions (lack thereof) amounted to a
substantial procedural violation within the meaning of Rule 67, first sentence, EPC.

IX. The respondent's submissions in writing and at oral proceedings may be summarized as follows:

Main and first auxiliary requests: claim 33
Article 84 EPC; clarity

- In claim 33 of the main request, the bacterial cells were characterised by the feature that they could not be transformed by being made competent.

  (i) None of the documents cited by the appellant provided evidence that the term "competence" would be given any other meaning than that of "natural competence". In particular, it would not be taken as meaning "artificial competence" as this term was known as being another way to refer to "artificial transformation".

  (ii) Natural competence was defined in essentially the same manner in documents D15 and D16 as the ability for the cell to bind and to take up DNA. The conditions to be used to achieve a state of competence were known (documents D3, D4). The fact that some authors could not find conditions to make certain strains competent (document D18) did not imply that the term was unclear.

- The reference to natural competence in claim 33 of the first auxiliary request was clear for the reasons given in (ii) above.
Second auxiliary request; claim 33
Articles 123(2) EPC and 84 EPC; support in the application as filed, clarity

- The claimed bacterial cell was defined as that "which has not been made by transformation of naturally competent cells". The application as filed disclosed many non-naturally competent starting strains so there could not be any problems under Article 123(2) EPC with regard to the added feature.

- The skilled person would have no difficulties in understanding that this process feature was in fact meant to characterize the starting strain for the transformation as not being naturally competent. The skilled person would not think that it could mean that the claimed bacterial cell was obtained by some other means than transformation as this could only be a theoretical possibility.

Third auxiliary request; claim 33
Article 84 EPC; lack of clarity

The claimed bacterial cell was defined as belonging to the genus Bacillus in addition to not being transformable by being made competent. At the priority date, the conditions to be used to make a Bacillus strain competent were well known as shown in document D3 or D4. There were no examples in the art of a Bacillus strain which would have been found non-competent and then competent later on. Document D18 was not relevant as it was published in 1964 ie well before the above mentioned reliable conditions for inducing competence in Bacilli were found.
**Article 56 EPC; inventive step**

**Claim 12**

Document D2 was concerned with studying the stability of a DNA of interest *when integrated* in a bacterial genome and not with getting a DNA of interest to integrate in a stable manner in said genome. The described in situ construct had a deficient origin of replication and it was taught that in situ replication should not occur if the insert was to remain stable. Taken together, these two teachings would lead the skilled person to delete the origin of replication. On the contrary, the method of claim 12 required that a functional origin of replication be present on the plasmid carrying the DNA of interest in order to ensure its transfer into the cells.

Document D1 described, in particular, how one plasmid may help another to replicate autonomously in the bacterial cytoplasm. There was mention neither of DNA integration into the bacterial genome nor of a potential instability of inserted DNA.

The skilled person would have no reasons to combine the teachings of documents D2 and D1 and even if they were combined, the skilled person would not arrive in an obvious manner to the method of claim 12.

**Claims 1 and 25**

The subject-matter of claim 1 was inventive for the same reasons as given in relation to the subject-matter of claim 12. Document D10 could not be damaging to the inventive step of the plasmid of claim 25: it did not
even show that the rolling circle mode of replication necessarily resulted in two different daughter plasmids. Still less did it show that the daughter plasmids if produced would be those of claim 25.

Claim 33

The appellant had failed to provide evidence that any methods other than the method of claim 12 would lead to the isolation of the non-competent bacterial cell of claim 33.

Indeed, document D4 like document D2 was concerned neither with non-competent cells nor with the transfer and integration of a DNA of interest into the bacterial genome. Rather, it showed that an integrated DNA was more stable at 51°C (when replication does not occur) than at 37°C (when it does). This teaching could not lead to the concept which permitted the isolation of the claimed bacterial cell.

The subject-matter of claim 33, which was the result of a new and inventive process, was patentable.

Rule 68 EPC; procedural violation

The decision of the opposition division was correct: in saying that the prior art was absolutely silent about the invention, the opposition division clearly meant not only the prior art was not novelty destroying but also that it did not comprise disclosures which would have led in an obvious manner to the invention. There was no need for the opposition division to address the matter in any lengthy way.
X. The appellant (opponent) requested that the decision under appeal be set aside, that the patent be revoked and that the appeal fee be reimbursed.

As main request, the respondent (patentee) requested that the appeal be dismissed. As auxiliary requests 1 to 4, the respondent requested that the decision under appeal be set aside and the patent be maintained with the claims of one of these auxiliary requests filed with letter dated 30 December 2002, taken in their numerical order. With respect to auxiliary request 3, the respondent also requested to maintain the patent with a description as adapted during the oral proceedings.

Reasons for the Decision

Main request; claim 33

Article 123(2)(3) EPC

1. The only amendment which was carried out after grant is in claim 33 where the bacterial cells are now characterised amongst other features as "which cannot be transformed by being made competent". The amendment, which was not challenged by the appellant under Article 123(2)(3) EPC, finds a basis on page 11, lines 11 to 16 of the application as filed. It amounts to a restriction of the scope of the claim. The requirements of Article 123(2)(3) EPC are fulfilled.

Article 84 EPC; clarity

2. According to Article 84 EPC, the claims shall be clear, concise and be supported by the description. Moreover,
in view of their importance for determining the extent of the protection conferred (Article 69(1) EPC), they must be clear for the sake of legal certainty (inter alia T 337/95, OJ EPO 1996, 628, points 2.2 to 2.5).

3. The appellant argued that there were two possible interpretations of the word "competent", namely "naturally competent" and "artificially competent" Document D20 which represents the common general knowledge before the priority date of the patent teaches natural and artificial transformation: artificial transformation corresponds to the physical treatment of the cells so that they become artificially competent i.e. capable of taking up DNA (document D20, page 202, lines 5 to 7); natural transformation amounts to growing the cells to such a stage that they become naturally competent i.e. capable of taking up DNA without further treatment (document D20, page 202, third paragraph). Artificial transformation is discussed in particular in the passage bridging pages 205 and 206; it is stated that calcium treatment "appears to be almost universally applicable amongst bacteria" and also, that other artificial means have been developed.

4. Thus, in the board's judgment, the skilled person aware from document D20 of the overall applicability of artificial transformation would have discarded the possibility that the amendment in claim 33 included artificial competence as this interpretation would not make any technical sense. Accordingly, no ambiguity is seen in the wording of claim 33 insofar as it can only be read as directed to bacterial cells which cannot be transformed by being made "naturally competent". This finding is in agreement with the established practice of the Boards of Appeal, see for example T 190/99 of

5. For the sake of legal certainty, the requirement of clarity is particularly important. When a claim is directed to a product which is characterized by parameters, the established jurisprudence requires that those parameters must be clearly and reliably determined by objective procedures which are usual in the art (T 94/82, OJ EPO 1984, 75). The same criteria applies to functional features. These features must provide instructions which are sufficiently clear to reduce them to practice without exceeding the normal skills and knowledge of the skilled person (inter alia T 68/85 OJ EPO 1987, 228 in particular point 8.4.3).

6. Claim 33 encompasses all species and even genuses of bacteria. Yet, there is no evidence on file that at the priority date, there existed a standard method for assessing in a clear and reliable manner the ability of bacteria in general to take up DNA from their surrounding. In fact, it is readily apparent from document D20 (page 202) that to become competent is not only an intrinsic physiological ability of the bacteria but also depends on growth conditions. This necessarily means that, in case bacteria are found to be non-competent, the skilled person is left in doubt as to the reason for this observation: whether it is because they cannot be transformed by being made competent, or because the proper experimental conditions for the induction of competence have not been found. Otherwise stated, it is not possible to identify the claimed bacteria with any certainty.

7. For this reason, the board concludes that claim 33 is not clear. The main request, which comprises this
First auxiliary request (ARI); claim 33
Article 84 EPC

8. The first auxiliary request differs from the main request in that the amendment in claim 33 reads: "... which cannot be transformed by being made naturally competent, and ...". The presence of the word "naturally" in this amendment does not confer any additional feature to the bacterial cell of claim 33 which would be susceptible to change the conclusion of lack of clarity reached for the reasons given in point 6 above. Therefore, the first auxiliary request does not meet the requirements of Article 84 EPC.

Second auxiliary request (ARII); claim 33
Article 123(2) EPC and Article 84 EPC; added subject-matter, clarity

9. The wording "a bacterial cell which has not been made by transformation of naturally competent cells " in claim 33 can be understood as meaning that transformation was not the experimental procedure used to obtain said bacterial cell. There is neither formal nor technical support in the contested patent for this feature. Accordingly, neither the requirement of Article 123(2) EPC, nor those of Article 84 EPC are fulfilled and the second auxiliary request is rejected.

Third auxiliary request (ARIII); claim 33
Article 123(2)(3) EPC

10. This request is identical to the main request except...
that claim 33 has been limited to bacteria cells: "... belonging to the genus Bacillus and which cannot be transformed by being made competent, and ...". No objections have been raised by the appellant under this Article. The amendment finds a basis on page 11, lines 11 to 19 of the application as filed as well as in the given examples. It amounts to a restriction of the scope of the granted claim. Thus, the requirements of Article 123(2)(3) EPC are met.

Article 84 EPC

11. Several documents of the prior art refer to well-established methods for inducing natural competence in bacterial cells belonging to the genus Bacillus. Documents D3 (page 116, right-hand column, second full paragraph) and D4 (page 40, left-hand column, last paragraph) refer to the method of Niaudet and Ehrlich (1979), whereas document D5 (page 4822, right-hand column, third full paragraph) refers to the method of Dubnau and Davidoff-Abelson (1971). Admittedly, all these methods are concerned with Bacillus subtilis. However, document D20 (page 203, Figure 13), which discloses the mechanism of natural competence at a more detailed genetic level, explicitly refers to Bacillus spp as being intensively studied and as the paradigm of Gram-positive transformation (by natural competence) (page 202, last sentence of the second full paragraph).

12. In view of the availability of standard methods as well as of detailed information at a genetic level (document D20), the board is satisfied that it is possible to differentiate between a Bacillus which can be made naturally competent and a Bacillus which cannot. In contrast to the situation described in point 6 above,
the skilled person can evaluate an occasional failure in the light of the prior art and come to a conclusion as to whether this failure is due to the conditions used for inducing competence or else to the inability of the bacteria to be made competent. Thus, a Bacillus which cannot be transformed by being made competent may be identified without exceeding the normal skills and knowledge of the skilled person. The subject-matter of claim 33 is clear.

13. Document D18, concerned with the transformation of B. licheniformis, was cited by the appellant as showing that even for bacteria belonging to the genus Bacillus, the skilled person can never be sure whether a failure to transform the bacteria is due to non-competence or else to a lack of success in finding the right conditions for inducing competence (page 525, left-hand column, second sentence under "Discussion"). However, document D18 is a publication dating from 1964 ie from at least seven years before the well-established methods referred in point 11 above were published and almost twenty years before the general disclosure of document D20 (point 11 above). In view of this other more pertinent prior art, document D18 looses relevance.

14. For these reasons, the third auxiliary request is considered to fulfill the requirements of Article 84 EPC.

Article 54 EPC

15. No objections of lack of novelty were raised on appeal. The board does not see any prior art on file disclosing the subject-matter of any of the claims under
consideration. Thus, the request satisfies the conditions laid down in Article 54 EPC.

**Article 56 EPC**

**Claim 12**

16. The closest prior art to the method of claim 12 is document D2. This document (page 2653, paragraph bridging right- and left-hand columns) discloses the transformation of a bacterial cell (B. subtilis JH648) with a ligated mixture of an integrational plasmid (pHV551, Figure 1) and a pheA segment. The resulting transformed strain, B. subtilis HVS552, carries in its genome the integrated non-replicative DNA construct comprising (1) a DNA sequence of interest (chloramphenicol resistance marker, CmR), (2) a DNA sequence homologous with a region of the genome of the cell (pheA segment), and (3) an origin of replication (ori) (pC194 ori), but lacking a functional gene coding for a factor required to initiate replication from said ori (truncated repA gene). The frequency of recombination between the pheA segments in the chromosome which represents the stability of the insert is $1.6 \times 10^{-4}$ (Table 2). In a second experiment (page 2654, right-hand column, second full paragraph), HVS552 is transformed with the plasmid pHV1020 carrying the DNA segment encoding the repA protein. The frequency of recombination between the duplicated pheA segments increases 100-fold. The authors attribute this decrease in stability of the integrated plasmid to the provision of the replication protein A in trans.

17. Thus, starting from this closest prior art, the objective technical problem may be defined as the provision of an alternative method of producing a
bacterial cell which carries in its genome a stable DNA construct. In view of the disclosure and examples of the contested patent, the board is convinced that the method of claim 12 provides a solution to this problem.

18. The difference between the method of claim 12 and the method used for obtaining HVS552 in document D2 resides in the fact that the former method requires to carry out the transformation using simultaneously two plasmids (one having the same relevant features as pHV551 and the other having the same relevant features as plasmid pHV1020) which are replicated before any integration takes place.

19. That the claimed method results in the stable integration of the DNA of interest in the genome could not be expected from the teaching of document D2 which shows that when the two plasmids are in the same bacterial cell, instability increases (see point 16 above). Admittedly, in the experimental setting disclosed in document D2, one of the plasmids is already integrated into the genome whereas the other replicates autonomously in the bacterial cytoplasm. Yet, in the board's judgment, the fact that a different experimental setting was developed in which the same tools (the replicating and the non-replicating plasmid) which were known from document D2 to increase instability when put together, now, on the contrary, enable stable integration to take place renders the claimed subject-matter non-obvious over the teaching of document D2 alone. It should also be mentioned that the claimed method has advantages over that disclosed in document D2 because the DNA of interest is amplified by replication in the cytoplasm (patent-in-suit, page 7).
20. The appellant argued that the combined teachings of documents D2 and D1 rendered obvious the subject-matter of claim 12. It was considered that, starting from document D2, the problem to be solved could be defined as how to replicate an integrative plasmid into a cell once it had entered and before integration into the genome. The view was expressed that solving this problem was obvious in light of document D1 which taught that a functional replication factor from one plasmid could act in trans to replicate a second plasmid.

21. Document D1, indeed, describes the Bacillus strain KY104 which is transformed with two plasmids. Plasmid pFTB91 has an ori (ori-14, active at 45°C) and a gene encoding a replication factor, whereas in plasmid pMM55, the ori which is functional in Bacillus, namely ori-194, is temperature sensitive ie active at or below 37°C but inactive at 45°C. Plasmid pMM55 does not carry a gene encoding a replication factor (page 293, Figure 1 and page 295, last full paragraph). By selecting transformants at 45°C, it is shown that the replication factor of pFTB91 is able to replicate plasmid pMM55.

22. There is nothing in document D1 to suggest that having a replicating and a non-replicating vector in the same cell may ultimately be advantageous to insert the non-replicating plasmid into the chromosome, let alone in a stable manner. In fact, document D1 does not even mention integration. In the same manner, as can be seen from point 16 above, document D2 is not concerned with the conditions to be met for a non-replicating and a replicating vector to be autonomously replicating in the bacterial cytoplasm because the replicating vector
pHV1020 described on page 2654 of said document is only transformed into the bacterial cell once the non-replicating vector is already integrated in the genome.

23. For these reasons, it is readily apparent that the problem to be solved cannot be defined from document D2 as the appellant did and that it is only with the hindsight knowledge of the claimed invention that documents D2 and D1 could be combined.

24. The board thus concludes that the subject-matter of claim 12 is inventive.

Claims 1 and 25

25. The difference between the method of claim 12 and that of claim 1 is that the latter makes use of one vector, namely the parental plasmid vector, which combines the features of the two DNA vectors used in the method of claim 12. Upon entry into the bacterial cell and after the transformants so obtained are grown under selective conditions, the parental plasmid vector gives rise to the two DNA vectors of claim 12. This feature does not alter the reasoning presented in points 16 to 19 above leading to the conclusion that the method of claim 12 is inventive. Accordingly, inventive step is also acknowledged to the subject-matter of claim 1.

26. The parental plasmid vector of claim 25 owes its characteristics to the fact that it is especially suited for carrying out the inventive method of claim 1. Accordingly, its structure is not derivable in an obvious manner from the prior art.

Claim 33
27. Document D2, which represents the closest prior art, discloses bacteria belonging to the genus Bacillus (B. subtilis HVS550 and HVS552, Figure 1) which are naturally competent and carry in their genome a stable, non-replicative DNA construct (pHV550 or pHV551).

28. Starting from the closest prior art, the objective technical problem is seen as the provision of further bacterial cells belonging to the genus Bacillus which carry in their genome a stable, non-replicative DNA construct. The provision of Bacillus bacteria which cannot be transformed by being made competent and carry this construct solves this technical problem.

29. Document D2 refers to several recombinant bacterial strains. However, all of them are B. subtilis strains (page 2654, Table 1) ie strains which are capable of becoming competent and no hint is given that the teachings relative to Bacillus subtilis could be applied to other bacteria belonging to the genus Bacillus, let alone to Bacillus strains which cannot be transformed by being made naturally competent. In this respect, the appellant has referred to document D12 as providing such a pointer, in particular to the sentence on page IV-2, second full paragraph: "Many of the plasmid vectors developed for use in B. subtilis can be used in other Bacilli, including B. pumilis, B. licheniformis and B. megaterium". It is argued that this teaching would prompt the skilled person to obtain transformed bacteria as described in document D2 starting from any Bacillus species. The board notices, however, that there is no reference in document D12 to either competence or non-competence. In the absence of such a reference, the question must be asked whether or not, at the priority date, the skilled person would
consider obvious on the basis of further prior art to apply the teachings of document D2, in particular to the claimed subset of Bacilli strains which cannot be transformed by being made competent.

30. At the priority date, it was already known from document D20 (page 203) that the transformation of bacteria by natural competence occurs by attachment of double stranded DNA to the cell membrane followed by the entry of one single strand which becomes integrated into the bacterial chromosome. It was also known (see reference to de Vos and Venema, 1981 in document D16, to be taken as an expert document) that the artificial transformation which must be used in case the bacterial cells are non-competent does not follow the same mechanism. In this case, the plasmid vector enters the cell as double stranded DNA. ssDNA is only formed by replication and, thus, integration can only occur if replication takes place (document D4, page 47, left-hand column, last paragraph). In the board's judgment, the skilled person aware of the teachings of documents D20 and D16 could not have any expectation of success of isolating the claimed non-competent bacterial cells by merely using the integrational non-replicative plasmid vectors of document D2 (pHV550 or pHV551). The subject-matter of claim 33 is inventive over the teachings of said document.

31. In the appellant's opinion, the subject-matter of claim 33 also lacks inventive step over the teachings of document D3. Document D3 describes B. subtilis competent cells transformed with a plasmid (pHV1405i) which is unable to replicate (interruption of the gene encoding the RepA protein necessary for replication of pC194 ori) and which is integrated into the bacterial
genome (page 117, Table 1 and Figure 1; page 118, left-hand column, first full paragraph). By induction of natural competence, the integration takes place in a straightforward manner without requiring any replication. In the board's judgment, this disclosure is no more relevant than the one of document D2 and, thus, does not affect inventive step for the same reasons (see points 16 to 19).

32. Finally, the appellant argued that knowing from document D4 that replication could be regulated by using a thermosensitive origin of replication, the skilled person would find it obvious to obtain the claimed bacteria: ie to transform the non-competent cells artificially with an integrative plasmid thermosensitive for replication, to allow replication to occur at the low temperature (ie to allow the formation of single-stranded DNA), to integrate the ssDNA into the bacterial genome and to increase the temperature so that replication would stop and the integrated DNA would be stable.

33. Document D4 indeed discloses the transformation of B. subtilis HVS224 competent cells by integrative plasmids which are thermosensitive for replication (pE194 cop-6, pHV1211). The transformants are said to be obtained at 51°C since the plasmid replication is not functional at that temperature. Tables 2 and 3 (pages 43 and 45) show that the plasmid DNA remains stably integrated in the bacterial chromosome at high temperature ie when no replication takes place but is unstable at low temperature when replication does take place. Otherwise stated, document D4 discloses bacterial strains in which the stability of the insert depends on the growth conditions.
34. Thus, like document D2, document D4 is not concerned with non-competent cells. Furthermore, the method which it describes is not suited for obtaining non-competent bacterial cells such as claimed i.e. which carry stable inserts in their chromosome irrespective of the growth conditions. In the board's judgment, it does not make the claimed subject-matter obvious. Thus, there is no evidence that any other methods than the inventive methods of claims 1 or 12 could lead to the isolation of the bacterial cells of claim 33.

35. In the Board's judgement, there are no other documents on file which could affect inventive step, whether taken on their own or in combination with document D2. For these reasons the subject-matter of claim 33 fulfills the requirements of Article 56 EPC.

36. No objections have been raised to the amended description (amended page 2) which was adapted to the patentable claim request.

Rule 68(2) EPC, Rule 67 EPC; procedural violation, refund of the appeal fee.

37. According to Rule 67, first sentence, EPC the reimbursement of appeal fees shall be ordered where the Board of Appeal deems an appeal to be allowable, which is the case here, provided such reimbursement is equitable by reason of a substantial procedural violation.

38. The appellant has submitted that in the context of its decision to acknowledge inventive step the opposition division had erroneously stated that the appellant had raised no objections against claims 33 to 39.
Furthermore, by basing this decision only on its finding that the novelty of the claimed subject-matter was given, the decision of the opposition division lacked the reasoning prescribed by Rule 68(2), first sentence, EPC. This amounted to a substantial procedural violation within the meaning of Rule 67, first sentence, EPC.

39. The above first-mentioned objection of the appellant could be taken to mean that the opposition division had violated the appellant's right to be heard within the meaning of Article 113(1) EPC. However, when reading the criticised sentence in the appealed decision in its proper context, which is the discussion of inventive step of amended claim 33 which had been limited to bacterial cells which cannot be transformed by being made competent ("non-competent cells"), thereby excluding the competent bacterial cells known from the prior art, it becomes clear that the opposition division intended to express with the criticised sentence that the appellant had not submitted a specific argumentation as to why the limited claim was still obvious in relation to the state of the art, ie even in view of its limitation to non-competent cells. As reasons for the alleged incorrectness of the opposition division's behaviour the appellant only submitted that the appellant's original objections to claim 33 as unamended which also extended to lack of inventive step, were not removed by the amended main request. It can however be derived from the reasoning of the opposition division on inventive step in point 4.2 that the opposition division was, on the contrary, of the opinion that the limitation of the teaching of claim 33 to non-competent cells was the decisive element rendering claim 33 non-obvious.
40. The second objection of the appellant relates to Rule 68(2), first sentence, EPC. According to said rule decisions of the European Patent Office which are open to appeal shall be reasoned.

41. Deficiencies of the appealed decision according to Rule 68(2), first sentence, EPC have been recognised in the jurisprudence as being procedural defects which may constitute substantial procedural violations rendering the reimbursement of the appeal fee equitable within the meaning of the said rule (Case Law of the Boards of Appeal of the European Patent Office, 4th edition 2001, VII.D.15.4.4). In order to satisfy the said provision it is, however, sufficient if the crucial points for the case are dealt with in a manner enabling the parties to understand the line of argument followed by the decision-taking body (T 75/91 of 11 January 1993, point 7 of the reasons), in order to give the party a fair idea of why his submissions were not considered convincing (T 740/93 of 10 January 1993, point 5.4 of the reasons) and to enable the party to understand whether the decision was justified or not (T 292/90 of 16 November 1992, point 2 of the reasons).

42. As regards the issue of inventive step of claim 33 the opposition division indicated in point 4.2 of the reasons for the decision that the stable integration of non-replicative DNA in the bacterial genome by transformation of "non-competent" bacterial cells was considered as non-obvious and thus inventive, since the prior art was absolutely silent with regard to the stable integration of a desired DNA into the chromosome of this particular group of bacterial cells. A further technical reason was added, ie these cells normally show only a low transformation frequency. Previously,
in the context of the discussion on novelty, the opposition division had pointed out that there was nothing in the documents cited by the appellant to suggest transformation of "non-competent" cells.

43. It is to be acknowledged that the reasoning in the appealed decision relating to inventive step of claim 33 is rather short. However, beside the fact that amended claim 33 was only one of several claims the opposition division had to consider, what is required by Rule 68(2), first sentence, EPC is not length as such. The reasoning must be such that the parties can derive from it which facts and arguments were decisive for the decision. As has been said above, in the present case it can clearly be derived from the opposition division's reasoning that it regarded the limitation of the teaching of claim 33 to "non-competent" cells as the decisive element of the claim rendering it non-obvious in relation to the state of the art. In the view of the board the formulations used by the opposition division indicate more than simply that the features of claim 33 were not disclosed in the prior art. When it is said that they were not "suggested" and even that "nothing" suggested the claimed subject-matter in the limited form of amended claim 33, and that the prior art was "absolutely silent" on the claimed transformation of non-competent cells these are formulations which indicate that in the view of the opposition division there was nothing in the prior art which could have led the skilled person to the claimed subject-matter, ie suggested the transformation of "non-competent" cells. This is a typical reasoning concerning a conclusion on inventive step. The opposition division has also given a further technical reason therefor, even if in very short form,
ie the low transformation frequency of the non-
competent cells used as starting materials. It can be
derived therefrom that the opposition division regarded
the case with respect to amended claim 33 as so clear-
cut that more comprehensive reasoning was not required
for this issue, by contrast with the reasoning on
inventive step of the method claims to which more room
was given. As has been stated in decision T 177/98 of
9 November 1999, point 5 of the reasons, it is the task
of the decision-taking body to grasp the relevant
arguments and to reply to them and in this context to
make a choice of the importance of the arguments, in
particular where more material has been provided, which
is the case here.

44. In conclusion, even if the reasoning of the opposition
division with respect to the acknowledgement of
inventive step of claim 33 is rather short and could
perhaps have been more comprehensive, the decisive
grounds for regarding said claim as inventive have been
indicated by the opposition division in a clear way.
There is therefore no lack of sufficient reasoning in
the appealed decision within the meaning of Rule 68(2),
first sentence, EPC, and also therefore no substantial
procedural violation within the meaning of Rule 67,
first sentence, EPC.

Order

**For these reasons it is decided that:**

1. The decision under appeal is set aside.

2. The case is remitted to the Opposition Division with
the order to maintain the patent with the claims of auxiliary request 3, filed with letter dated 30 December 2002, amended page 2 of the description, filed during the oral proceedings, pages 3 to 15 of the description and Figures 1 to 31 as granted.

3. Reimbursement of the appeal fee is refused.

The Registrar: The Chairman:

A. Wolinski F. Davison-Brunel