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**Datasheet for the decision
of 10 November 2020**

Case Number: T 1092/17 - 3.3.08

Application Number: 10779999.1

Publication Number: 2436777

IPC: C12Q1/68, G01N21/64

Language of the proceedings: EN

Title of invention:

METHOD FOR DETECTING VARIATIONS IN NUCLEIC ACID SEQUENCES

Patent Proprietor:

Xiamen University

Opponent:

STRAWMAN LIMITED

Headword:

Self-quenched probe/XIAMEN UNIVERSITY

Relevant legal provisions:

EPC Art. 84, 123(2)

RPBA Art. 12(4)

Keyword:

Main request, 1st and 2nd auxiliary request - added matter -
(yes)
3rd to 5th auxiliary requests - not admitted
6th to 11th auxiliary requests - admitted - (yes)
6th to 8th auxiliary requests - added matter - (yes)
9th to 11th auxiliary requests - clarity - (no)

Decisions cited:

T 0842/14

Catchword:



Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 1092/17 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 10 November 2020

Appellant:

(Patent Proprietor)

Xiamen University
No. 422 Si Ming Nan Road
Siming District
Xiamen, Fujian 361005 (CN)

Representative:

Held, Stephan
Meissner Bolte Patentanwälte
Rechtsanwälte Partnerschaft mbB
Widenmayerstraße 47
80538 München (DE)

Respondent:

(Opponent)

STRAWMAN LIMITED
Orchard Lea
Horns Lane
Combe
Witney, Oxfordshire OX29 8NH (GB)

Representative:

Schlörb, Christian
Vossius & Partner
Patentanwälte Rechtsanwälte mbB
Siebertstrasse 3
81675 München (DE)

Decision under appeal:

**Decision of the Opposition Division of the
European Patent Office posted on 24 February
2017 revoking European patent No. 2436777
pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman D. Rogers
Members: M. R. Vega Laso
 P. Julià

Summary of Facts and Submissions

- I. The appeal of the patent proprietor (appellant) lies from a decision of an opposition division posted on 24 February 2017, revoking the European patent No. 2 436 777 with the title "Method for detecting variations in nucleic acid sequences". The patent was granted from the European application No. 10779999.1 which was filed under the Patent Cooperation Treaty and published as WO 2010/135917 A1. In the following, references to the "the application as filed" are to the English translation of the application, published in accordance with Article 153(4) EPC on 4 April 2012.
- II. The patent was opposed on the grounds for opposition of Article 100(a) in conjunction with Articles 54 and 56 EPC, and Article 100(b) and (c) EPC.
- III. In the decision under appeal, the opposition division found that the subject-matter of claim 1 of each of the main request and auxiliary requests 1 and 2 extended beyond the content of the application as filed and, thus, contravened Article 123(2) EPC.
- IV. Together with the statement setting out the grounds of appeal, the appellant re-filed the sets of claims according to the main request and auxiliary requests 1 and 2 underlying the decision under appeal as, respectively, main request, 1st and 2nd auxiliary request in appeal, and submitted three additional sets of claims as 3rd to 5th auxiliary requests.
- V. Claim 1 according to the **main request** reads as follows:

"1. A method for detecting the presence of a variation or the type of a variation in a target nucleic acid, comprising:

(1) preparing a probe directed to the nucleic acid target(s) where the detection of nucleic acid sequence variation is needed;

wherein the probe is a self-quenched nucleic acid probe, wherein the probe is labeled at opposite ends with a fluorescent group and a quenching group in such a way that fluorescence or fluorescence intensity increases when the probe hybridizes with the target nucleic acid sequence compared to fluorescence or fluorescence intensity in the absence of the target nucleic acid sequence, wherein: if the 5' end of the probe is labeled with the fluorescent group, then the 3' end of the probe is labeled with the quenching group; or if the 3' end of the probe is labeled with the fluorescent group, then the 5' end is labeled with the quenching group, and

said probe does not comprise a modification that is able to resist the exonuclease activity of a polymerase, and said probe is a linear probe, and wherein the length of the probe is 5-100 bases;

(2) amplifying a fragment (or fragments) comprising the nucleic acid(s) to be tested using asymmetric PCR, wherein one PCR amplification primer in the reaction mixture is relatively in excess and the strand produced with the elongation of said primer hybridizes with the probe and the polymerase used in the asymmetric PCR has an exonuclease activity, and the melting temperature of the probe is not lower than the melting temperature of the primer used for PCR amplification; and adding said probe into the amplification reaction before the amplification;

(3) after the amplification, performing melting curve analysis for the amplification product comprising

said probe added in step (2); determining whether the nucleic acid(s) to be tested has a variation and optionally the possible type of variation, based on the corresponding melting temperature detected by the self-quenched probe (or the melting temperature of the hybrid formed between said probe and the nucleic acid sequence to be tested), or differences of said melting temperature."

Dependent claims 2 to 13 are directed to various embodiments of the method of claim 1.

- VI. Claim 1 of the **1st auxiliary request** differs from claim 1 of the main request in that the polymerase used in the asymmetric PCR in step (2) of the claimed method is a "*Taq polymerase having an exonuclease activity*".
- VII. Claim 1 of the **2nd auxiliary request** differs from claim 1 of the 1st auxiliary request in that the wording "*... said probe does not comprise a modification that is able to resist the exonuclease activity of a polymerase ...*" in step (1) of the method has been replaced by "*... said probe consists of unmodified bases ...*".
- VIII. Claim 1 of the **3rd auxiliary request** differs from claim 1 of the main request in that the polymerase used in the asymmetric PCR in step (2) has "*a reduced exonuclease activity*".
- IX. In claim 1 of the **4th auxiliary request** the polymerase is specified to be a "*TaqFS polymerase having a reduced exonuclease activity*".
- X. Claim 1 of the **5th auxiliary request** includes the amendments introduced in the corresponding claim of the

2nd and 4th auxiliary requests (see sections VII and IX above).

In each of the 1st to 5th auxiliary requests dependent claims 2 and 3 have been amended in the same manner as the corresponding claim 1. Claims 4 to 13 are identical to those of the main request.

- XI. The opponent (respondent) replied to the statement of grounds.
- XII. In reply to the respondent's submissions, the appellant submitted further sets of claims as 6th to 11th auxiliary requests, and documentary evidence.
- XIII. The sets of claims according to the **6th to 11th auxiliary requests** differ from those of the main request and 1st to 5th auxiliary requests in that the features "*wherein the length of the probe is 5 to 100 bases*" and "*the melting temperature of the probe is not lower than the melting temperature of the primer used for PCR amplification*" have been deleted in the respective claims 2 and 3.
- XIV. Pursuant to their respective subsidiary request, the parties were summoned to oral proceedings before the board.
- XV. In a communication issued in preparation of the oral proceedings, the board drew attention to matters which seemed to be of special significance and expressed a provisional opinion on some procedural and substantive issues, in particular issues relating to Article 123(2) EPC.

XVI. Oral proceedings were held on 10 November 2020 in the presence of both parties.

XVII. Following document is cited in this decision:

(I): L. T. Parker *et al.*, 1996, *Biotechniques*,
Vol. 21, No. 4, pages 694 to 699.

XVIII. The submissions made by the appellant, as far as they are relevant to the present decision, were essentially as follows:

*Main request, 1st and 2nd auxiliary request -
Article 123(2) EPC*

The opposition division erred in finding that the application as filed did not provide a direct and unambiguous disclosure for the combined use of a non-modified probe with a polymerase having exonuclease activity. Claim 19 of the application as filed disclosed a method using a probe according to claims 1 to 17. In paragraph [0036] of the application as filed it was indicated that "generally said self-quenching probe consists of common bases" and it was explicitly stated in the first sentence of paragraph [0042] that appropriate "reaction conditions" could - and had to - be chosen for maintaining the integrity of the probe. Whilst it was also contemplated in paragraph [0037] that the self-quenched probe may comprise modifications, in particular modifications which enable resistance against exonuclease activity, none of the specific modifications that could be implemented to this purpose (such as locked nucleic acids (LNA) or functional group modifications in the form of, e.g., phosphorothioated linkages) was mentioned in the context of Example 6.

Example 6 showed that, using a probe without modification, when appropriate reaction conditions were chosen, namely "asymmetric PCR", results were always obtained - regardless of the polymerase used. As shown in Figure 6 and stated in paragraph [0082], the results were the same using either the Taq polymerase or the TaqFS polymerase, the former with a standard/normal exonuclease activity and the latter with reduced activity.

In contrast, in Example 1 (see paragraph [0058]) the linkage between the first and second base in the 5' end of probe 1 was said to be a phosphorothioated linkage, i.e. probe 1 was explicitly disclosed as a modified probe. Also the probes in Examples 3 and 4 were described as LNA-modified probes (see paragraphs [0067] and [0075]). Since a unmodified probe was the general case, and Example 6 did not mention the presence of a modification, the skilled person would have derived from Example 6 that the linear self-quenched probe therein was not modified.

3rd to 5th auxiliary requests - Article 12(4) RPBA

The 3rd to 5th auxiliary requests could not have been filed in opposition proceedings. The specific reasons given in the decision under appeal for the revocation of the patent had been totally unexpected. Prior to the decision, the opposition division had not expressed its concerns about Article 123(2) EPC, nor given the patent proprietor the chance to file amended claims. The request should be admitted into the proceedings.

6th to 11th auxiliary requests - Article 12(4) RPBA

The requests had to be considered by the board because they had been filed in due time in response to objections raised by the respondent in appeal.

6th to 8th auxiliary requests - Article 123(2) EPC

The arguments put forward in connection with claim 1 of the main request and the 1st and 2nd auxiliary request were relevant also to these requests.

9th auxiliary request - Article 84 EPC

The term "*reduced exonuclease activity*" had a sufficiently recognized meaning in the art and was clear within the meaning of Article 84 EPC. Moreover, the use of relative terms in a claim was not absolutely prohibited by the EPC. For determining whether the exonuclease activity of a given polymerase was reduced, the exonuclease activity of the *Taq* polymerase represented the reference value.

10th and 11th auxiliary request - Article 84 EPC

The term "*TaqFS*" had a well-recognized meaning in the art and did not designate a trademark. The term designated a *Taq* polymerase in which the phenylalanine in position 667 was replaced by tyrosine. It was common general knowledge in the art that *TaqFS* has a reduced exonuclease activity.

XIX. The relevant submissions by the respondent were as follows:

*Main request, 1st and 2nd auxiliary request -
Article 123(3) EPC*

The decision of the opposition division was correct. The combination of features in claim 1 of each request was not disclosed in the application as filed. Whilst claim 19 of the application as filed was directed to a method for detecting the presence of a variation or the type of a variation in a target nucleic acid, several selections and combinations had to be made for arriving at the features characterising the claimed method, in particular for those characterising the probe. Relevant for the definition of the probe were claims 20 and 21 of the application as filed, wherein a link was made between (modified/non-modified) probe and the exonuclease (standard/reduced or low) activity of the polymerase used. That definition and link were in line with disclosure in the description of the application as filed, wherein no preference/prioritisation was made between modified and non-modified probes (paragraph [0027]) but, if at all, more emphasis on the former as shown in paragraphs [0036], [0037] and [0042]. Indeed, it was in the light of all these disclosures and the teaching conveyed to the reader/skilled person that Example 6 had to be read.

Example 6 was not a basis for the subject-matter of claim 1 of the main request because this example related to a specific experiment using specific primers, probes, polymerases, buffer conditions and experimental parameters. None of these features was part of claim 1. Regardless of the reaction conditions, symmetric or asymmetric PCR, the information given in paragraph [0082] and Figure 6 was that two *Taq* polymerases, one with standard exonuclease activity and the other with reduced activity, were available to the

skilled person and that they had to be selected in line with the whole teaching of the application. Moreover and in any case, none of the specific reaction conditions mentioned in paragraph [0081] was present in the claimed method. Hence, the claimed subject-matter extended beyond the content of the application as filed.

3rd to 5th auxiliary requests - Article 12(4) RPBA

The grounds for which the opposition division had revoked the patent had been well known to the appellant, and it had had the opportunity to file amended claims with its observations on the notice of opposition. Moreover, the requests did not remedy the deficiencies of the earlier requests and/or introduced new problems under Articles 84, 123(2) and 56, and Rule 80 EPC.

6th to 11th auxiliary requests - Article 12(4) RPBA

For the same reasons as for the 3rd to 5th auxiliary requests, these requests should not be admitted into the proceedings.

6th to 8th auxiliary request - Article 123(2) EPC

The same objections as for the main request and the 1st and 2nd auxiliary request applied.

9th to 11th auxiliary request - Article 84 EPC

The term "reduced" in claim 1 was a relative term which did not have a well-defined meaning and which was also not defined in the patent, e.g. by way of a reference value and threshold. Therefore, it lacked clarity and contravened the provisions of Article 84 EPC.

The term "*TaqFS*" in claim 1 lacked clarity. The patent did not provide any source or definition for this polymerase. The reference to be taken for interpreting the term "reduced" when in connection with *TaqFS* was ambiguous and not clear whether the wording "*TaqFS polymerase having a reduced exonuclease activity*" meant that a *TaqFS* polymerase was modified in order to have a reduced activity when in comparison to a non-modified *TaqFS* polymerase, or that the activity of the *TaqFS* polymerase was already "reduced" because it was compared to the standard *Taq* polymerase.

- XX. The appellant (patent proprietor) requested that the decision under appeal be set aside and the case be remitted to the opposition division for examination of the further grounds for opposition, based on any of the main request and the 1st to 11th auxiliary requests.
- XXI. The respondent (opponent) requested that the appeal be dismissed. The respondent also requested that the 3rd to 5th and 9th to 11th auxiliary requests not be admitted into the appeal proceedings. Subsidiarily, the respondent requested that the case be remitted to the opposition division for further consideration of the grounds for opposition of Article 100(a) EPC.

Reasons for the Decision

Main request, 1st and 2nd auxiliary request - Article 123(2) EPC

1. The claims of the present main request and 1st and 2nd auxiliary request are identical to those of the corresponding requests underlying the decision under appeal.

2. In the decision under appeal, the opposition division found that the application as filed does not provide a clear and unambiguous disclosure of the subject-matter of claim 1 of each of these requests, in particular as the claimed methods involve the use of a polymerase having exonuclease activity **and** a self-quenched probe which does not comprise a modification able to resist the exonuclease activity of the polymerase (see points 7.5 and 8.1 of the decision under appeal).
3. The appellant contested these findings. As a basis for the subject-matter of claim 1 of the main request, the appellant pointed to claim 19 of the application as filed, which refers to claims 1 to 17 defining the self-quenched probe, in combination with paragraph [0036] of the description allegedly providing a clear disclosure of a method that generally involves the use of a self-quenched probe which consists of common bases, i.e. a non-modified self-quenched probe.
4. The board does not share this view. Even though some of the features of the method of the present claim 1, in particular those characterizing the probe are disclosed in claims 1 to 17 and claim 19 of the application as filed, in order to arrive at the method of claim 1 several selections and combinations of disclosed features would have to be made, without any pointer in the application as filed. Hence, the subject-matter of claim 1 cannot be considered to be directly and unambiguously derivable from a combination of claim 19 with claims 1 to 17 of the application as filed.
5. As regards the appellant's reference to paragraph [0036] of the application as filed as a basis for a method in which the probe does not comprise a

modification that is able to resist the exonuclease activity of the used polymerase, the board observes that the application as filed discloses in paragraphs [0036] to [0040] various modifications of a self-quenched probe used in different embodiments of the method of the invention, including modified bases, modified linkages between bases, and structural modifications, like a hairpin structure. Whilst paragraph [0036] describes modifications aimed at modulating (i.e. increasing or decreasing) the binding activity of the probe, the relevant disclosure of modifications of the probe in order to maintain its integrity when a polymerase having exonuclease activity is used, is found in paragraphs [0037] to [0039].

6. Paragraph [0037] of the application as filed reads:

"In a preferred embodiment, when using a DNA polymerase having a 5'→3' exonuclease activity for the PCR amplification, the self-quenched probe can be modified to resist the 5'→3' exonuclease activity of the DNA polymerase; when using a DNA polymerase having a 3'→5' exonuclease activity for the PCR amplification, the probe can be modified to resist the 3'→5' exonuclease activity of the DNA polymerase probe. Thus, during the entire amplification reaction, the integrity of the probe is maintained, making it possible to perform subsequent hybridization reaction and melting curve analysis."

7. A person skilled in the art derives from this passage that, since it is essential for carrying out the method described in the application to maintain the integrity of the self-quenched nucleic acid probe during the initial amplification reaction, if a DNA polymerase

having either 5'->3' or 3'->5' exonuclease activity is used for amplification, the probe needs to be protected against enzymatic digestion by the DNA polymerase. To this purpose, paragraph [0037] of the application discloses that the self-quenched probe is modified to resist the 5'->3' or 3'->5' exonuclease activity of the DNA polymerase. Specific modifications which render the probe able to resist the exonuclease activity of the polymerase are disclosed in paragraphs [0038] and [0039].

8. Contrary to appellant's view, in the light of the disclosure in the application as a whole, the wording "*can be modified*" in paragraph [0037] would not be understood by the skilled person as meaning that also a probe which does not comprise a modification that can resist the exonuclease activity of a polymerase, could be used in combination with a polymerase having such activity. Such an interpretation would be at odds with following statements in paragraph [0042] of the application as filed:

"... when the probe itself lacks the ability of resisting the 5'- and 3'-exonuclease activity of the enzyme, thermostable nucleic acid polymerases lacking the 5'- and 3'-exonuclease activity, such as KlentTaq, may be used; alternatively, thermostable nucleic acid polymerases having very low 5'-exonuclease activity and no 3'-exonuclease activity, such as Taq FS, may be used."

9. On this account, the board shares the opposition division's view that the disclosure in the passage of the application as filed indicated by the appellant does not provide a basis for the method of claim 1.

10. The appellant relied also on the disclosure in Example 6 of the application as filed and contested the opposition division's finding that this example does not provide a clear and unambiguous disclosure as to whether or not the probe used in the experiment is modified (see point 7.4 of the decision). Referring to the explicit disclosure of modified probes in Examples 1, 3 and 4, the appellant argued that, since a modification is not explicitly mentioned in Example 6, the skilled person understands that the probe used in the experiment together with a polymerase having exonuclease activity (*Taq*), is not modified.

11. Even if the board accepted appellant's argument that the skilled person would derive from the application that the probe used in Example 6 is not modified, the board is nevertheless unable to find in Example 6 a clear and unambiguous disclosure of a probe which, **specifically**, "*... does not comprise a modification that is able to resist the exonuclease activity of a polymerase*", as recited in claim 1 of the main request and the 1st auxiliary request. This wording excludes modifications that render the probe able to resist the exonuclease activity of the polymerase, but does not exclude other types of modification, e.g. modifications resulting in an increase or decrease of the binding activity. Hence, the alleged disclosure of an unmodified probe in Example 6 cannot serve as basis for the more specific feature in claim 1.

12. Moreover, as regards appellant's argument that, regardless of the polymerase used, results are always obtained for asymmetric PCR, the board considers that the specific reaction conditions used in Example 6 may result in a sufficient amount of intact probe for melting curve analysis after the PCR reaction, but

claim 1 neither comprises all these reaction conditions nor requires this latter feature.

13. For these reasons, a method as defined in claim 1 of both the main request and the 1st auxiliary request extends beyond the disclosure in Example 6 of the application as filed.
14. As regards the 2nd auxiliary request, the disclosure in Example 6 of a specific, allegedly unmodified probe of 26 nucleotides in length (see page 16, line 13 of the application and SEQ ID NO:58 of the Sequence Listing) cannot be regarded as a basis for a probe which consists of unmodified **bases** and has a length of **5-100** bases, as recited in claim 1. Hence, also the subject-matter of claim 1 of the 2nd auxiliary request extends beyond the content of the application as filed.
15. In view of the findings above, the board concludes that the subject-matter of claim 1 of each of the main request and the 1st and 2nd auxiliary requests is not directly and unambiguously derivable from either the general disclosure or the more specific disclosure in Example 6 of the application as filed. Hence, these requests contravene Article 123(2) EPC.

3rd to 5th auxiliary requests - Article 12(4) RPBA

16. The respondent requested that the sets of claims of the 3rd to 5th auxiliary requests not be admitted into the proceedings. These sets of claims were filed together with the statement of grounds of appeal, in response to the allegedly surprising findings of opposition division on Article 123(2) EPC in the decision under appeal.

17. However, the issues addressed by the amendments introduced into the claims of the 3rd to 5th auxiliary requests had been raised already in sections 2.3 and 2.4 of the notice of opposition to substantiate the ground for opposition of Article 100(C) EPC. Thus, these sets of claims could - and should - have been filed already in response to the notice of opposition. Hence, the board, exercising its discretionary power, decided to disregard these requests (Article 12(4) RPBA).

6th to 11th auxiliary requests - Article 12(4) RPBA

18. The board concurs with the appellant that these requests were filed as a reaction to an objection under Rule 80 EPC raised for the first time in appeal. As they are part of the appeal proceedings, the board took these requests into account for its decision on the appeal.

6th to 8th auxiliary requests - Article 123(2) EPC

19. The sets of claims of the 6th to 8th auxiliary requests were filed in response to an objection under Rule 80 EPC raised by the respondent for the first time in its reply to the grounds of appeal. Hence, they could not have been presented in opposition proceedings. Consequently, they are taken into account by the board for its decision on the appeal.

20. Claim 1 of each of the 6th to 8th auxiliary requests is identical to claim 1 of, respectively, the main request and the 1st and 2nd auxiliary requests. Hence, the reasons given in paragraphs 4 to 12 above apply, *mutatis mutandis*, equally to the 6th to 8th auxiliary

requests. Thus, also these requests offend against Article 123(2) EPC.

9th auxiliary request - Article 84 EPC

21. In claim 1 of this request, the polymerase used in the amplification step of the claimed method is characterized as "*having a reduced exonuclease activity*". It was not disputed by the appellant that the term "reduced" is a relative term, and that the patent provides neither a definition nor a standard level of exonuclease activity for comparison.
22. According to the case law of the Boards of Appeal (see, *inter alia*, decision T 842/14 of 23 January 2019), a claim cannot be regarded as clear within the meaning of Article 84 EPC if it comprises a technical feature for which no unequivocal generally accepted meaning exists in the art. Whilst the appellant contended that the feature "*having a reduced exonuclease activity*" had a generally accepted meaning in the art, it did not provide any evidence to this effect, even though an objection under Article 84 EPC had been raised by the respondent in this respect.
23. The board concurs with the respondent that the feature "*having a reduced exonuclease activity*" is vague and does not define clearly the polymerase used for amplification. As this technical feature of the polymerase is an essential feature for performing the method of claim 1, and is also highly relevant to the assessment of novelty and inventive merit of the claimed subject-matter, the board considers the clarity requirement of Article 84 EPC not to be fulfilled.

10th and 11th auxiliary requests - Article 84 EPC

24. Claim 1 of each of these requests specifies that the polymerase used for amplification is "*TaqFS polymerase having a reduced exonuclease activity*".
25. The term "*Taq FS*" appears in the patent as an example of a thermostable nucleic acid polymerase having "*very low 5'-exonuclease activity and no 3'-exonuclease activity*" (see paragraph [0060]), and a "*TaqFS enzyme having reduced exonuclease activity*" is used as polymerase for amplification in the experiment described in Example 6 of the patent (see paragraph [0096]), but the patent provides no information on the chemical nature of the enzyme or the commercial source of the enzyme preparation.
26. In reply to the respondent's clarity objection to the term "*TaqFS*", the appellant submitted document (I) allegedly showing that *TaqFS* is a well-known mutant of the *Taq* polymerase and part of the common general knowledge in the art. Document (I), which is a research report in a specialized journal, cannot, however, be considered a sufficient evidence for the common general knowledge of a person skilled in the art.
27. The board shares the respondent's view that, besides the uncertainty as to the nature of *TaqFS*, the wording of the feature at issue leaves open whether a "*reduced exonuclease activity*" is an intrinsic characteristic of *TaqFS*, or an additional feature required for performing the claimed method. In the latter case, the clarity issue discussed above in connection with the 9th auxiliary request arises.

28. In view of the above, the wording of claim 1 does not allow to ascertain for which subject-matter the appellant is seeking protection. Hence, the requirement of Article 84 EPC is not met.

Conclusion

29. Since the amendments introduced into the sets of claims on file either contravene Article 123(2) EPC (main request and 1st, 2nd and 6th to 8th auxiliary requests) or give rise to objections under Article 84 EPC (9th to 11th auxiliary requests), there is no set of claims on file on the basis of which the patent could be maintained.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairman:



L. Malécot-Grob

D. Rogers

Decision electronically authenticated