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Datasheet for the decision of 2 May 2023

Case Number: T 2701/19 - 3.3.08

Application Number: 11075256.5

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Language of the proceedings: EN

Title of invention:
Methods and compositions for DNA manipulation

Patent Proprietor:
New England Biolabs, Inc.

Opponent:
HGF Limited

Headword:
DNA manipulation/NEW ENGLAND BIOLABS

Relevant legal provisions:
EPC Art. 56, 113(1)
RPBA Art. 12(4)
RPBA 2020 Art. 13(1)

Keyword:
Main request and auxiliary request 1 and 2 - inventive step - (no)
Auxiliary request 3 - late filed facts and objection - admitted (no)

Decisions cited:
T 0197/10, T 0217/15, T 0442/15, T 0169/20

Catchword:
-

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Case Number: T 2701/19 - 3.3.08

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

Appellant I: (Patent Proprietor)	New England Biolabs, Inc. 240 County Road Ipswich, MA 01938 (US)
Representative:	Griffin, Philippa Jane Mathys & Squire The Shard 32 London Bridge Street London SE1 9SG (GB)
Appellant II: (Opponent)	HGF Limited Saviour House 9 St Saviour Gate York YO1 8NQ (GB)
Representative:	Taylor, Kate Laura HGF HGF Limited 1 City Walk Leeds LS11 9DX (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 2 August 2019 concerning maintenance of the European Patent No. 2455488 in amended form.**

Composition of the Board:

Chair	P. Julià
Members:	B. Claes
	A. Bacchin

Summary of Facts and Submissions

I. The appeals lodged by the patent proprietor (appellant I) and by the opponent (appellant II) lie from the interlocutory decision of the opposition division that European patent No. 2 455 488 (the patent) as amended according to auxiliary request 3, which was filed during the oral proceedings in the opposition proceedings, and the invention to which it relates meet the requirements of the EPC. The title of the patent is "*Methods and compositions for DNA manipulation*".

II. The opposition proceedings were based on the grounds for opposition under Article 100(a) EPC, here in relation to novelty (Article 54 EPC) and inventive step (Article 56 EPC), Article 100(b) and 100(c) EPC. In the decision under appeal the opposition division held, *inter alia*, that the subject-matter of the set of claims of auxiliary requests 1 and 2 lacked an inventive step.

III. Unless indicated otherwise, any reference to the Rules of Procedure of the Boards of Appeal (RPBA) in this decision is to the version of the RPBA which entered into force on 1 January 2020 (OJ EPO 2022, supplementary publication no. 1, III.2), with the amendment that came into force on 1 April 2021 (OJ EPO 2021, A35), and which apply to any appeal case pending on that date (Articles 24(1) and 25(1) RPBA). Pursuant to Article 25(2) RPBA, the new Article 12(4) to (6) does not apply to appeals where the statement of grounds of appeal was filed before 1 January 2020 and any reply thereto filed in due time. Instead, Article

12(4) RPBA 2007 continues to apply. This is the case for the appeals in the present case.

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

D6: WO03/012100 (A2) (publication date: 13 February 2003);

D10: Krokan *et al.*, "DNA glycosylases in the base excision repair of DNA", Biochem. J., vol. 325, 1997, pages 1 to 16;

D11: Extract from "Boehringer Mannheim 1998 Biochemicals Catalog";

D12: Extract from "USB 1999-2000 Catalog";

D13b: "Epicentre: BESS T-Scan™ Mutation Detection and Localization Kit", 3 July 1998 (Available at www.epicentre.com/lit/besslit.htm#excision);

D13f: Hawkins *et al.*, "Simple, Rapid, Nucleotide-Level Characterization of Sequence Variations Using the BESS T-Scan™ Mutation Detection and Localization Kit" in: Epicentre Forum, vol. 4., No. 2, 1997, pages 1 to 4;

D14: Extract from "New England Biolabs 2000-01 Catalog & Technical Reference";

D15: Extract from "MBI Fermentas 2000-2001 Molecular Biology Catalog & Product Application Guide";

D16: Extract from "Life Technologies. Catalogue and

Reference Guide 2001";

D17: Extract from "New England Biolabs 2002-03 Catalog & Technical Reference";

D18: Summary of the enzyme products identified in documents D11 to D17 (1998 to 2002);

D25: US 6,333,178 (B1) (publication date: 25 December 2001).

V. Appellant I submitted with its statement of grounds of appeal a set of claims according to a main request (which is identical to the set of claims according to the version of auxiliary request 1 that was addressed in the decision under appeal) and sets of claims according to auxiliary requests 1, 2 and 3 (the latter two being identical to those addressed in the decision under appeal). Appellant I also submitted arguments in favour of the allowability of each of these requests.

Claims 1 to 18 of the main request and of auxiliary requests 1 and 2 are method claims. Claim 19 of the main request and claim 19 of auxiliary request 1 are identical and read as follows:

"19. A nicking agent that is capable of excising a modified nucleotide from a polynucleotide molecule, wherein the nicking agent is a reagent comprising a mixture of two or more enzymes, at least one of which is a DNA glycosylase and at least one of which is a single-strand cleaving enzyme;

wherein the DNA glycosylase is UDG glycosylase; and

wherein the single-stranded cleaving enzyme is

selected from FPG glycosylase/AP lyase and EndoVIII glycosylase/AP lyase."

Claim 19 of auxiliary request 2 is identical to claim 19 as above but for the deletion of the wording "selected from FPG glycosylase/AP lyase and".

Auxiliary request 3 comprises only method claims; all product claims (nicking agent) have been deleted.

VI. Appellant II argued in its statement of grounds of appeal that the subject-matter of the set of claims of auxiliary request 3 lacked an inventive step and submitted three new documents.

VII. Each party replied to the appeal of the other party.

VIII. In accordance with the requests of the parties, the board summoned the parties to oral proceedings and, in a subsequent communication pursuant to Article 15(1) RPBA, informed them of its preliminary opinion on various matters concerning the appeal.

IX. Prior to the scheduled date for oral proceedings, both appellants withdrew their request for oral proceedings without making any further substantive submissions on the board's preliminary opinion. The board therefore decided to cancel the oral proceedings.

Appellant I's submissions, where relevant to this decision, can be summarised as follows:

Main request - claim 19

Claim construction

The claimed nicking agent was a reagent that comprised a mixture of a DNA glycosylase enzyme and a DNA glycosylase/AP lyase, combined in the same buffer and "*ready to use*". For the skilled person a *reagent* was an "*off-the-shelf*" product that necessarily had a certain shelf life and was formulated so as to maintain enzyme stability until use in any reaction where the properties of that reagent were required. Thus, when interpreting the term "nicking agent" in the light of paragraph [0057] of the patent, a reagent was not a "pre-mix" or "master-mix" formed by combining a DNA glycosylase and a DNA glycosylase/AP lyase enzyme at the last minute prior to adding these enzymes to a reaction.

Inventive step (Article 56 EPC)

Document D25 represented the closest prior art. The method disclosed in Example 3 involved the *separate* supply, storage, measurement and addition to the reaction mixture of the enzyme uracil DNA N-glycosylase (UDG) and the enzyme MuTM glycosylase/AP lyase (FPG). The distinguishing feature of the claimed nicking agent was therefore that it was pre-formed and comprised a mixture of a DNA glycosylase enzyme and a DNA glycosylase/AP lyase enzyme.

The objective technical problem was to provide an improved (e.g. more efficient) means of using a DNA glycosylase enzyme and a DNA glycosylase/AP lyase enzyme for excising a modified nucleotide from a polynucleotide substrate.

The fact that a common reaction buffer for the UDG reaction step and the FPG reaction step was disclosed in document D25 in a reaction lasting only 30 minutes

did not prompt the skilled person to combine these two enzymes into a "nicking agent" reagent as claimed, i.e. in the same storage buffer where long-term stability was required. In fact, the purpose of a reaction buffer was different from that of a storage buffer and they typically had different compositions.

DNA glycosylase enzymes and DNA glycosylase/AP lyase enzymes were always sold separately, each formulated in a different storage buffer. Document D18 summarised the different storage and reaction buffers used in documents D11 to D17 for UDG and various DNA glycosylase/AP lyase enzymes, including FPG. Document D18 confirmed that i) in each case UDG was supplied in a different storage buffer from the DNA glycosylase/AP lyase enzymes, and ii) DNA glycosylase enzymes and DNA glycosylase/AP lyase enzymes were supplied in different storage buffers at different concentrations. Furthermore, it was not suggested in the art to combine a DNA glycosylase enzyme and a DNA glycosylase/AP lyase enzyme into a "master-mix" (or any other "pre-mix") prior to addition to a substrate in a reaction. Accordingly, "*in light of the prevailing practice and opinion in the art away from combining these enzymes*", the skilled person had no reasonable expectation that the activities of a DNA glycosylase enzyme and a DNA glycosylase/AP lyase enzyme would be retained when these enzymes were combined in the same buffer to form a nicking agent. The skilled person would therefore not have contemplated combining the DNA glycosylase and the FPG enzyme in the same buffer to form a nicking agent as claimed.

The skilled person would not have turned to document D13f, as its disclosure had no relevance for solving the objective problem. In fact, the enzyme

mixes disclosed in this document contained an enzyme with DNA glycosylase activity (FPG) and the single-strand cleaving enzyme "EndoIV", the latter being an AP endonuclease enzyme and not a DNA glycosylase/AP lyase enzyme as referred to in the claim. Document D13f did not provide any guidance on how to improve excision using the specific combination of a DNA glycosylase and a DNA glycosylase/AP lyase enzyme.

The combined teaching in these documents would not have led the skilled person to the claimed nicking agent with a reasonable expectation of not impairing enzyme activity given the very limited information in document D13f, the functional and structural differences between EndoIV and the DNA glycosylase/AP lyase enzymes, and the prevailing opinion in the art teaching away from combining a DNA glycosylase and a DNA glycosylase/AP lyase into a single reagent. The EndoIV disclosed in document D13f belonged to a different enzyme super-family of single-strand cleaving enzymes which differed considerably in function and structure from AP lyases. In view of their functional and structural differences, the skilled person would not have expected that enzymes from these different super-families would behave in the same way when combined with a DNA glycosylase (e.g. UDG) in a nicking agent. Accordingly, the skilled person would not have combined the two enzymes into a pre-mix; rather, they would have expected that this would lower or even abolish the activity.

Auxiliary request 2 - claim 19 - inventive step

Example II of the patent described in paragraph [0149] an assay to determine the optimal amount of EndoVIII in a nicking agent reagent containing 0.2 activity units

of UDG. The results demonstrated (see Figures 23 to 25) that complete digestion of the dU-containing substrate occurred with at least 31.25 ng of EndoVIII and 0.2 units of UDG - i.e. 1 activity unit of "USER™ Enzyme" could be prepared by mixing at least 31.25 ng of EndoVIII with 0.2 units of UDG. In contrast, paragraph [0150] reported that complete digestion of the same substrate under the same experimental conditions required at least 145 ng of FPG and 0.1 units of UDG - i.e. 1 unit of "USER™ Enzyme 2" could be prepared by mixing at least 145 ng of FPG and 0.1 units of UDG (i.e. at least 290 ng FPG per 0.2 units of UDG). Accordingly, the patent demonstrated that considerably less EndoVIII than FPG was required in order to cleave the same AP-containing substrate to completion. Thus, EndoVIII was more efficient than FPG at cleaving AP sites generated by UDG in a polynucleotide substrate. The findings in Example II of the patent were confirmed by later-published evidence.

Starting from the disclosure in document D25, the objective technical problem facing the skilled person was thus to provide a more efficient way of excising a modified nucleotide from a polynucleotide substrate using UDG glycosylase and a DNA glycosylase/AP lyase. Document D25 did not suggest replacing FPG with any other DNA glycosylase/AP lyase, let alone EndoVIII.

Although FPG and EndoVIII were both DNA glycosylase/AP lyase enzymes and shared several regions of significant sequence similarity, FPG was "more distantly related" than other DNA glycosylase/AP lyase enzymes (see document D10, Figure 8). As there was no suggestion in the state of the art of the improvement in efficiency observed in the patent when using EndoVIII instead of FPG, the skilled person was not motivated to modify the

excision method described in Example 3 of document D25 in such a way as to use EndoVIII instead of FPG.

In light of the state of the art, the skilled person had no reasonable expectation that UDG and EndoVIII could be combined in a reagent in the same buffer without loss of enzyme activity.

Auxiliary request 3 - inventive step

Admittance of a new line of attack and three new documents into the appeal proceedings

In its statement of grounds of appeal, appellant II had solely argued, and this for the first time during the proceedings, that the claimed subject-matter lacked an inventive step in view of the disclosure in document D6, which represented the closest prior art, and the disclosure in document D13b, and that document D6 should be interpreted in light of the "common general knowledge" disclosed in three newly-filed documents. In accordance with Article 12(4) RPBA, this new submission should not be admitted or taken into consideration in the appeal proceedings.

Appellant II's submissions, where relevant to this decision, can be summarised as follows:

Main request - claim 19

Claim construction

The definition of the term "nicking agent" in paragraph [0057] of the patent did not impose any limits on the storage time or conditions required of a nicking agent. The claim therefore covered all pre-

mixing of enzymes, including the preparation of a pre-mix immediately prior to addition to a reaction.

Inventive step (Article 56 EPC)

The disclosure in document D25 represented the closest prior art and the distinguishing feature of the claimed nicking agent was that it was pre-formed by mixing the DNA glycosylase and the DNA glycosylase/AP lyase (prior to addition into a reaction buffer with a polynucleotide substrate having a modified nucleotide).

The objective technical problem was to provide an improved means of using a DNA glycosylase and DNA glycosylase/AP lyase enzyme for excising a modified nucleotide from a polynucleotide substrate.

The claimed invention allowed the preparation of a pre-mix of these enzymes at any time prior to the addition thereof to a polynucleotide substrate in a reaction buffer. The claim was silent with regard to a buffer and stability. Moreover, there was no evidence that the skilled person would have assumed a loss of enzyme activity when these enzymes were combined.

The concurrent use in a single reaction mix of a DNA glycosylase together with a single-strand cleaving enzyme, without any evidence of a detrimental impact on function, was routine in the art (see, e.g., documents D6 and D25). There was no prevailing opinion in the art that taught away from combining DNA glycosylases and single-strand cleaving enzymes. Documents D11 to D17 taught the skilled person that UDG could be stored in a variety of buffers, at a variety of pHs. Documents D13 and D17 described single-strand cleaving enzymes provided in a variety of buffers, at a variety of pHs,

but did not suggest that a DNA glycosylase and a DNA glycosylase/AP lyase were not suitable for being mixed prior to addition to an assay.

The efficiency benefits, in terms of set-up, time savings and consistency, associated with the preparation of a "pre-mix" were known to the skilled person and it would be entirely obvious to attempt to improve the efficiency of the methods disclosed in document D25 by pre-mixing reagents prior to addition to a reaction mixture.

Document D13f described kits and methods for use in excising uracil nucleotides from a polynucleotide. The disclosure in document D13f of a functional nicking agent that combines in a single reagent a DNA glycosylase and a single-strand cleaving enzyme (AP endonuclease; EndoIV), thus motivated the skilled person to try other combinations of DNA glycosylases and single-strand cleavage enzymes, including the combination of the nicking enzymes used in the excision method disclosed in document D25, with a reasonable expectation of success.

Auxiliary request 2 - claim 19 - inventive step

The newly added distinguishing feature defines the claimed nicking agent as containing a mixture of UDG glycosylase and EndoVIII glycosylase/AP lyase enzyme as the single-strand cleaving enzyme instead of the single-strand cleaving enzyme *E. coli* MutM (FPG) protein used in document D25, and was unrelated to the requirement that the DNA glycosylase and the single-strand cleaving enzyme were pre-mixed. Accordingly, these differences related to a different partial problem to be solved.

The patent did not disclose any technical advantage associated with the further difference; EndoVIII was a known functionally equivalent alternative to FPG, and both enzymes were alternatives. There were no data available to demonstrate that the efficiency of the EndoVIII AP cleavage could be attributed to any synergistic combination of EndoVIII and UDG, rather than just the obvious selection of an alternative DNA glycosylase/AP lyase. As such, the replacement of one enzyme (FPG) for the other (EndoVIII) was a routine modification. The claimed subject-matter thus lacked an inventive step.

Auxiliary request 3 - inventive step

As submitted in point 2 of the statement of grounds of appeal (see section VI above)

The claimed subject-matter lacked an inventive step in view of the disclosure in document D6, representing the closest prior art, when interpreted in light of the common general knowledge as disclosed in the three newly-filed documents.

As submitted in point 5 of the reply to the statement of grounds of appeal of appellant I (see section VII above)

For all of the reasons discussed in respect of claim 2 of the main request, the subject-matter of claim 1 of auxiliary request 3 lacked an inventive step over the disclosure of document D6 in combination with that of document D13b, the latter document representing the closest prior art.

X. Appellant I requested that the decision under appeal be set aside and the patent be maintained on the basis of the set of claims of either, but in this order, the main request or auxiliary request 1 or 2 or, alternatively, that the appeal of appellant II be dismissed (i.e. that the patent be maintained on the basis of the set of claims of auxiliary request 3). Appellant I further requested that the new inventive step objections and the three documents (D29 to D31) filed by appellant II with the statement of grounds of appeal not to be admitted into the appeal proceedings.

Appellant II requested that the decision under appeal be set aside and the patent be revoked. Appellant II further requested that the three documents (D29 to D31) filed with the statement of grounds of appeal be admitted into the proceedings.

Reasons for the Decision

1. Both appeals are admissible.

2. Since both appellants withdrew their request for oral proceedings, the decision can be issued in writing without oral proceedings.

3. The decision is based on the same grounds, arguments and evidence on which the board's preliminary opinion was based (see section VIII) and on which the parties had the opportunity to present further comments and arguments (Article 113(1) EPC). These were not contested by the parties, nor did other aspects arise that would have required re-consideration.

Main request and auxiliary request 1

Scope of the appeal proceedings

4. The main request is identical to the set of claims of the version of auxiliary request 1 that was addressed in the decision under appeal. Thus, the main request already forms part of these proceedings.

5. Auxiliary request 1 is identical to the set of claims of auxiliary request 4, which was pending before the opposition division at the end of the oral proceedings and which in turn was identical to auxiliary request 1A, which was filed in writing on 10 May 2019 and renumbered at the end of the oral proceedings.

6. The decision under appeal did not formally address the current version of auxiliary request 1, because following the appellant I's renumbering of the auxiliary requests, the opposition division held the set of claims of auxiliary request 3 allowable.

7. The board acknowledges in this context that in principle the fact that a given claim request was submitted by a party in the opposition proceedings does not justify *automatically* admitting this claim request into the appeal proceedings (see, e.g., decisions T 442/15, Reasons 14, and T 217/15, Reasons 39.2). This is particularly the case when admittance of such a claim request has not even been examined by the opposition division and when a reasoned decision of the opposition division regarding the critical issues at hand has in effect been prevented by the party filing higher-ranking requests(in this case new auxiliary requests 2 and 3) during the oral proceedings.

8. The critical issue in the present case is, however inventive step of the subject-matter of claim 19, which is identical in both the main request and auxiliary request 1 (see section V.). Accordingly, the board has come to the following conclusions, which apply to both the main request and auxiliary request 1.

Claim 19 - claim construction

9. Paragraph [0057] of the patent reads as follows: "*The term "nicking agent" refers to a reagent which is capable of both recognizing a sequence-specific target, and nicking the target at a phosphodiester bond within or in a defined relationship to such sequence-specific target. The target comprises at least one nucleotide, where the nucleotide is a modified nucleotide*."

10. The board disagrees with appellant I that, in view of this definition and the reference therein to the term *reagent*, so-called "master-mixes" or "pre-mixes" prepared or formed at the last minute prior to addition into the reaction buffer are not covered by claim 19. In fact, the board sees no reason to use the description to interpret the term "nicking agent" or "agent", let alone to read in implicit restrictive features that are not suggested by the explicit wording of the claim (see, in particular, decisions T 197/10, Catchword, and T 169/20, Reasons 1.4, and Case Law of the Boards of Appeal of the European Patent Office, 10th edition, 2022, II.A.6.3.4, "CLBA"), e.g. that the claimed nicking agent must have a certain shelf life and must be formulated so as to maintain enzyme stability until use (i.e. a long, extended storage time) - as argued by appellant I.

11. The board thus agrees with the decision under appeal on this issue (see point 7.4 of the decision under appeal).

Inventive step (Article 56 EPC)

Closest prior art, distinguishing features, technical effect and objective technical problem

12. In line with the decision under appeal (see point 7.1) it is and was uncontested in the appeal and opposition proceedings, respectively, that the disclosure in Example 3 of document D25 of a method of excising a modified nucleotide from a plasmid molecule which contained in the reaction mixture a DNA glycosylase (i.e. uracil DNA N-glycosylase, also known as UDG) and an AP lyase (e.g. *E. coli* MutM, also known as FPG) represented the closest prior art.

13. Equally undisputed is the fact that the distinguishing feature of the claimed nicking agent is that the enzymes are in the form of a premixed formulation and are not added sequentially to the reaction. There is also consensus that, in view of the effects of this difference, such as being simpler, more efficient and less error prone, the objective technical problem can be formulated as the provision of an *improved* means of excising a modified nucleotide.

Obviousness

14. The opposition division held in the decision under appeal that when seeking to provide a solution for the objective technical problem, "*the skilled person would have been aware from his common general knowledge that the provision of a master-mix greatly enhances*

efficiency and reduces errors when pipetting several reactions in parallel" and that document D13f taught that a "Single Step" reaction was one of the "Ideal Criteria" for DNA-mutation scanning. The opposition division accordingly decided that, since document D25 already disclosed that the two enzymes - as referred to in claim 19 - were *present and active in a common reaction buffer* in the disclosed excision method (see column 32, lines 56 to 60, of document D25), it would have been obvious to the skilled person that the two enzymes can be pre-mixed before starting the reaction by the addition of substrate, i.e. in the form of the claimed "nicking agent" (see point 7.4 of the decision under appeal).

15. Appellant I submitted a number of lines of argument to substantiate its assertion that the opposition division's decision was wrong.

16. In a first line of argument, appellant I submitted that the purpose of an enzymatic reaction buffer was different from that of an enzyme storage buffer and that these buffers typically had different compositions. Thus, the use of a common reaction buffer in document D25 for the UDG and FPG reaction lasting only 30 minutes did not prompt the skilled person to combine these two enzymes into a "nicking agent" in one (single, common) storage buffer providing long-term enzyme stability.

17. The board refers to the claim construction in points 9. to 11. above, and notes that claim 19 does not require the claimed nicking agent to be in a form suitable for long-term stability, let alone to be formulated in a *storage* buffer as suggested by appellant I.

Accordingly, this first line of argument is not convincing.

18. In a second line of argument, appellant I submitted that document D18 demonstrated that various different storage buffers were used for supplying DNA glycosylase enzymes and DNA glycosylase/AP lyase enzymes. In view of the prevailing practice and opinion in the art teaching "*away from*" combining these enzymes, the skilled person would have had no reasonable expectation that the activities of a DNA glycosylase enzyme and a DNA glycosylase/AP lyase enzyme would be retained if these enzymes were combined in the same (single, common) buffer to form a nicking agent. The skilled person would therefore not have combined the DNA glycosylase and the FPG enzyme in the same (single, common) buffer to form a nicking agent as according to that of claim 19.

19. The board agrees with appellant I that document D18, which summarises the enzyme compositions disclosed in documents D11 to D17, reveals that commercial DNA glycosylase enzymes and DNA glycosylase/AP lyase enzymes are available in a variety of different storage buffers, at a variety of concentrations, and from a variety of different suppliers. However, the board is of the opinion, in agreement with appellant II but contrary to appellant I, that the data and information provided by document D18 *neither* support *nor* establish the existence of a technical prejudice to, nor teach away from, combining these enzymes in a pre-mix or in a "master-mix" as in the nicking agent of claim 19. Document D18 and the documents referred to in this document do in fact describe single-strand cleaving enzymes provided in a variety of buffers and at a variety of pHs, but they do not disclose that a DNA

glycosylase and a DNA glycosylase/AP lyase are not suitable for being mixed prior to addition to an assay. Accordingly, this line of argument is not convincing either.

20. In a third line of argument, appellant I submitted that the skilled person starting from the disclosure in document D25 and seeking to provide an improved means of using a DNA glycosylase enzyme and a DNA glycosylase/AP lyase enzyme for excising a modified nucleotide from a polynucleotide substrate would not have turned to document D13f, and even if the skilled person had done so, the combined teaching of these documents would not have led him/her to the claimed nicking agent with a reasonable expectation of success, i.e. without the loss of enzyme activity.

21. As to the first aspect of this line of argument, the board notes that document D25 and document D13f both relate to the excision of modified nucleotides from a polynucleotide and that the skilled person addressed in these documents is therefore the same. The argument that the skilled person would not have considered the disclosure in document D13f because it discloses excision methods using a nicking agent comprising a DNA glycosylase and a single-strand cleaving enzyme (AP endonuclease, in particular EndoIV), as opposed to a DNA glycosylase/AP lyase, is therefore not convincing.

22. As to the second aspect of this line of argument, the board is not convinced by appellant I's submission that, given the very limited information in document D13f, the substantial functional and structural differences between EndoIV and the DNA glycosylase/AP lyase enzymes, and the prevailing opinion in the art teaching away from combining a DNA

glycosylase and a DNA glycosylase/AP lyase into a single reagent (see above), the skilled person would not have arrived at combining the enzymes of document D25 in a pre-mix with the reasonable expectation that this would not impair enzyme activity; rather, the skilled person would have expected that this would lower or even abolish this activity.

23. However, the board has not seen any evidence in the relevant submissions of appellant I as to why or under what circumstances the skilled person would have (reasonably) expected the claimed combination of a DNA glycosylase and a DNA glycosylase/AP lyase to lower or abolish the activity of these enzymes. Thus, this aspect of the line of argument is also unconvincing.

24. In fact, the board agrees with appellant II that the disclosure in document D13f of a functional nicking agent that combines in a single reagent a DNA glycosylase and a single-strand cleaving enzyme (here the AP endonuclease EndoIV) would have led the skilled person to reasonably expect that combinations of DNA glycosylases and other single-strand cleavage enzymes (such as DNA glycosylase/AP lyase referred to in claim 19) could also be successfully pre-mixed before use - regardless of possible functional/structural differences among these single-strand cleavage enzymes.

25. In view of the above considerations, the board sees no reason to hold the decision that the subject-matter of claim 19 lacks an inventive step to be wrong. Thus, the main request does not fulfil the requirements of Article 56 EPC.

26. It follows that, as was also indicated in point 25 of the board's communication under Article 15(1) RPBA (see

section VIII. and point 3. above), the same conclusion applies to the identical subject-matter of claim 19 of auxiliary request 1. Thus, auxiliary request 1 does not fulfil the requirements of Article 56 EPC either.

Auxiliary requests 2 and 3 - scope of the appeal proceedings

27. Auxiliary requests 2 and 3 are identical to the set of claims of the versions of auxiliary requests 2 and 3 that were addressed by the opposition division in the decision under appeal. Thus, these auxiliary requests already form part of the proceedings.

Auxiliary request 2 - claim 19 - inventive step

28. As compared to the claim 19 of the main request, the wording "selected from FPG glycosylase/AP lyase and" has been deleted.

29. Starting from the disclosure in Example 3 of document D25, the further distinguishing feature of claim 19 is now - in addition to the difference applying also to the nicking agent of the main request, i.e. a pre-mixed "nicking agent" reagent - that the claimed nicking agent comprises a mixture of UDG glycosylase and *EndoVIII enzyme*, in particular the presence of this EndoVIII enzyme instead of the *E. coli* MutM (FPG) glycosylase/AP lyase described in the example (see document D25, column 32, lines 50 to 60).

30. The board agrees with appellant II that the two differences are unrelated and that each difference must therefore be considered as constituting a solution for a partial technical problem to be solved (see CLBA, I.D.9.3.2).

31. The opposition division noted that no particular technical effect was disclosed in the patent for the latter further difference. The partial problem relating to this further difference could thus only be the provision of an *alternative* method of excising a modified nucleotide. It was known from document D10 that FPG and EndoVIII were functionally equivalent AP lyases (see, e.g., Figure 2). The opposition division thus concluded that the further difference did *not* bestow the claimed subject-matter with an inventive step because it was obvious to the skilled person to replace the FPG used in D25 with EndoVIII as disclosed in document D10 (see point 10.2 of the decision under appeal).

32. Appellant I has not contested that FPG and EndoVIII were functionally equivalent AP lyases but has, however, referred to paragraphs [0149] and [0150] in Example II of the patent as allegedly demonstrating that EndoVIII was *more efficient* as the DNA glycosylase/AP lyase than FPG when used in combination with UDG in a nicking agent reagent of the invention, because less *weight amount* of EndoVIII glycosylase/AP lyase was required as compared to FPG (see USER™ Enzyme and USER™ Enzyme 2 in paragraphs [0149] and [0150], respectively, of the patent).

33. On the basis of the submissions of appellant I, however, it is inconceivable to the board why the nominal weight amount difference of the two DNA glycosylase/AP lyase enzymes in the USER™ Enzyme and USER™ Enzyme 2 demonstrate a difference in terms of efficiency at cleaving AP sites generated by UDG in a polynucleotide substrate in the absence of the molecular weight of these enzymes (see point 27 of the board's communication pursuant to Article 15(1) RPBA).

The board therefore sees no reason to hold the opposition division's decision that no particular technical effect was disclosed in the patent to be incorrect.

34. In view of the above considerations, the board is of the opinion that both the reasons of the opposition division and the decision that the subject-matter of claim 19 of auxiliary request 2 lacks an inventive step are correct.

The appeal of appellant I

35. In the light of the board's conclusions on the main request and auxiliary requests 1 and 2, it follows that the appeal of appellant I is to be dismissed.

The appeal of appellant II

Auxiliary request 3 - claim 1 - inventive step

36. In its statement of grounds of appeal, appellant II attacked solely this claim request, which was held allowable by the opposition division, asserting that the subject-matter of claim 1 lacked an inventive step in view of the disclosure in document D6, representing the closest prior art, in combination with the disclosure in document D13b. Three newly-filed documents were also relied on with this line of attack.

Admittance of a new line of attack and three new documents into the appeal proceedings

37. The board agrees with appellant I that the line of attack based on the disclosure in document D6 and the three newly-filed documents were submitted for the

first time in the appeal proceedings and, thus, this new line of attack is governed by Article 12(4) RPBA 2007. Indeed, in the opposition proceedings, the inventive-step attack relating to this request was based on document D13b representing the closest prior art.

38. Appellant II did not provide reasons for not submitting this new line of attack and the related documents until the appeal proceedings and, in particular, it did not argue that the new line of attack and the documents were filed, for example, in response to a new argument of the opposition division in the decision under appeal, to which they had no time to react.

39. Therefore, the board sees no reason to conclude that the new line of attack and the documents could not have been presented earlier and, thus, the board is of the opinion that they should have been filed during the opposition proceedings (Article 12(4) RPBA 2007).

40. Accordingly, as stated in the board's communication pursuant to Article 15(1) RPBA (see points 31 to 33), appellant I's request that the new inventive-step objections filed by appellant II - which are based on the disclosure in document D6 representing the closest prior art and the three newly filed documents - not be admitted into the proceedings is granted.

Admittance of a line of attack that had not been pursued with the statement of grounds of appeal

41. In the decision under appeal, the opposition division solely assessed the inventive step of this claim request based on a problem/solution approach starting from the disclosure in document D13b, which represented

the closest prior art, in combination with the secondary disclosure in document D6 (see point 12 *et seq*. of the decision under appeal).

42. With its appeal, appellant II did *not* submit that this part of the decision under appeal starting from the disclosure in document D13b was wrong; rather, it limited itself to submitting a new line of attack according to which the subject-matter of claim 1 lacked an inventive step starting from the disclosure of document D6 representing the closest prior art (see point 36. above). Appellant II, on appeal, was thus no longer pursuing the line of attack based on the disclosure in document D13b representing the closest prior art, with the consequence that, for this part, the appealed decision became final.

43. It was only with the reply to the appeal of appellant I (see section VII above), in the context of the claims of the current *main* request, that appellant II submitted arguments asserting that the subject-matter of claim 2 of the main request lacked an inventive step based on an analysis starting from the disclosure in document D13b representing the closest prior art in combination with the disclosure in document D6. In relation to claim 1 of auxiliary request 3, the reply contains merely the cursory remark, as an add-on in point 5, that for "*all of the reasons discussed above in respect of claims 2 as well as claims 15-18 of the MR, claim 1 (as well as claims 2-4 and 14-17) of AR3 lack an inventive step over the disclosure of D6 in combination with D13B*."

44. Accordingly, it was not until the reply stage of appellant I's appeal that appellant II, as the respondent to this appeal, submitted arguments as to

why the decision under appeal should be reversed with regard to this auxiliary request. However, Article 12(3) RPBA requires that the statement of grounds of appeal set out the reasons why the appellant is requesting that the decision under appeal be reversed.

45. The board holds that the introduction of the arguments based on the disclosure in document D6, representing the closest prior art in relation to claim 1 of auxiliary request 3, constitutes an amendment to appellant II's case, which may be considered at the board's discretion pursuant to Article 13(1) RPBA.

46. The board noted in the communication pursuant to Article 15(1) RPBA (see section VIII above) that it was unable to identify in appellant II's reply any reasons for introducing the arguments based on the disclosure in document D6 representing the closest prior art, which had not been pursued at the stage of filing the statement of grounds of appeal (see point 38 of the board's communication). In response to the board's communication, appellant II neither argued that the board had overlooked such arguments in its statement of grounds of appeal nor submitted arguments to justify the filing of the arguments at the reply stage.

47. Thus, in view of the current state of the proceedings and the circumstances in the case at hand, namely i) that no reasons have been provided for the late filing and ii) that the appellants have withdrawn their requests for oral proceedings in response to the board's communication pursuant to Article 15(1) RPBA (see section IX above), and with a view to further procedural economy, the board has decided neither to admit nor to consider appellant II's late-filed line of

argument in the appeal proceedings (Article 13(1) RPBA).

Conclusion

48. In view of the above considerations, the board has not seen any convincing arguments from appellant II that the decision of the opposition division, namely that the patent as amended in the form of the version of auxiliary request 3 that was filed during the oral proceedings in the opposition proceedings (and re-submitted in the appeal proceedings) and the invention to which it relates meet the requirements of the EPC, was wrong.

49. It follows that appellant II's appeal must be dismissed.

Order

For these reasons it is decided that:

Both appeals are dismissed.

The Registrar: The Chair:

C. Rodríguez Rodríguez P. Julià

Decision electronically authenticated