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**Datasheet for the decision
of 11 December 2017**

Case Number: T 1481/15 - 3.3.01

Application Number: 01924218.9

Publication Number: 1343877

IPC: C12N9/16, C12N9/22, C12Q1/68

Language of the proceedings: EN

Title of invention:
STABLE COMPOSITION COMPRISING A NUCLEASE AND A PHOSPHATASE

Applicant:
Affymetrix, Inc.

Headword:
Stable composition/AFFYMETRIX

Relevant legal provisions:
EPC Art. 56, 123(2)

Keyword:
Inventive step - (no) - technical prejudice in the art (no)
Amendments - allowable (no)

Decisions cited:
T 0332/12

Catchword:



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Case Number: T 1481/15 - 3.3.01

D E C I S I O N
of Technical Board of Appeal 3.3.01
of 11 December 2017

Appellant: Affymetrix, Inc.
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Decision under appeal: **Decision of the Examining Division of the
European Patent Office posted on 26 February
2015 refusing European patent application No.
01924218.9 pursuant to Article 97(2) EPC**

Composition of the Board:

Chairman A. Lindner
Members: T. Sommerfeld
M. Blasi

Summary of Facts and Submissions

- I. The appeal lies from the decision of the examining division whereby European patent application 01924218.9, based on an international application published as WO 01/70943, was refused under Article 97(2) EPC.

- II. A first decision of the examining division to refuse the patent application was appealed and set aside by decision T 332/12. In that decision Technical Board of Appeal 3.3.8 concluded that the claims of auxiliary request 9 filed with letter of 27 June 2013 fulfilled the requirements of Articles 123(2), 84 and 83 EPC and remitted the case to the examining division for further prosecution. In the course of further examination, the applicant, by letter dated 14 November 2014, filed a main request (identical to auxiliary request 9 of 27 June 2013) and a first auxiliary request, on the basis of which the examining division issued its second decision to refuse the patent application. It decided that the main request lacked inventive step (Article 56 EPC) and did not admit the auxiliary request into the proceedings (Rule 137(3) EPC).

- III. The applicant (hereinafter, the appellant) lodged an appeal against the examining division's decision, requesting that it be set aside and that a patent be granted according to the main claim request or to the auxiliary request, both filed with the statement of grounds of appeal.

- IV. By letter dated 6 July 2016, the appellant requested acceleration of the appeal proceedings.

- V. On 13 January 2017, the board sent a communication pursuant to Rule 100(2) EPC and Articles 12(1)(c) and 17(1) RPBA, in which it provided a preliminary opinion concerning inventive step for the main request and admission of the auxiliary request.
- VI. With letter of reply dated 12 May 2017, the appellant filed new first and second auxiliary requests.
- VII. With letter dated 14 November 2017, sent in reply to the summons to oral proceedings before the board, the appellant submitted an expert declaration (Horn's declaration, D7).
- VIII. Oral proceedings took place as scheduled. At the end of the oral proceedings the chairman announced the board's decision.
- IX. The **main request** comprises eight claims, claim 1 reading as follows:

"1. A composition comprising Exonuclease I and shrimp alkaline phosphatase, said composition being free from the presence of amplified deoxyribonucleic acid, wherein said composition further comprises at least 20 volume percent of a stabilizer selected from the group consisting of glycerol, ethylene glycol and glycine."

Claim 1 of the **first auxiliary request** differs from claim 1 of the main request in the addition of the following features:

"1. ..., a buffering agent, a reducing agent and a chelating agent."

Claim 1 of the **second auxiliary request** differs from claim 1 of the main request by the following amendments:

"1. A method of degrading preselected nucleic acids present in a sample of material, the method comprising the step of contacting said sample with a A composition comprising Exonuclease I, and shrimp alkaline phosphatase, at least 20 volume percent of a stabilizer selected from the group consisting of glycerol, ethylene glycol and glycine, a buffering agent, a reducing agent and a chelating agent, said composition being free from the presence of amplified deoxyribonucleic acid and has been stored at -20°C for 24 hours prior to contacting the sample., wherein said composition further comprises at least 20 volume percent of a stabilizer selected from the group consisting of glycerol, ethylene glycol and glycine."

X. The documents cited in the examination and appeal proceedings include the following:

- D1 WO 93/06243
- D2 Hanke M & Wink M 1994, BioTechniques, 17(5), 858-860
- D4 Olsen R et al. 1991, Comp. Biochem. Physiol., 99B(4), 755-761
- D5 Declaration by Mr Brock, dated 14 May 2015
- D6 Declaration by Mr Moffett, dated 5 December 2001
- D7 Declaration by Mr Horn, dated 13 November 2017

XI. The appellant's arguments which are relevant to the present decision may be summarised as follows:

The invention was about overcoming the prejudice in the prior art that Exonuclease I (Exo) and shrimp alkaline

phosphatase (SAP) could not be combined in one preparation because the EDTA present in the Exo preparation would inactivate the SAP. Once the prejudice was overcome by the inventors, then any formulation according to claim 1 would be suitable. In the prior art, it was considered "best practice" to add enzymes separately, as confirmed in the declaration of Dr Horn (D7, sections 4 and 5), an expert who had been working with these enzymes at the priority date together with D1's inventor (section 2); this was even more justified in the case of the enzymes disclosed in the application as filed, due to the presence of impurities and the consequent fear of unwanted cross-reactions (section 6). D4 confirmed the known prejudice by demonstrating that SAP was inhibited by EDTA (abstract 4.); activity restoration by addition of Zn^{2+} required complete removal of EDTA, which was however needed for Exo activity, and would not be an efficient way to proceed. The claiming of a composition of two enzymes always related to functional enzymes: this was inherent in the enzymes, and was also apparent e.g. from claim 19 of D1, where it was implicit that the claimed alkaline phosphatase should be active. There was no evidence that any of the buffers falling within the claim scope would not work. As to composition C, it was shown in Brock's declaration (D5) that the enzymes were active. While EDTA was considered indispensable for Exo I activity, the application showed that this was not the case (page 22, first paragraph). The problem was to provide a more efficient way to deal with several samples while avoiding pipetting errors, with fewer steps involved but with stability, consistency and accuracy in the results.

Regarding the first auxiliary request, the same arguments applied; the core agents of the formulations

tested, in particular of compositions A, D and E, were now part of the claim.

As regarded the second auxiliary request, a basis for the feature "has been stored at -20°C for 24 hours" could be found in the Examples, where each of the compositions had been stored for 24 hours or longer prior to contacting with the samples. All samples were stored at -20°C; there was a further basis on page 10, lines 7 to 9, and page 4, line 10, the 50% functional activity being inherent in all compositions comprising the components listed in the claim. It was implicit in the application that the purpose of the invention was to have stably storable solutions: e.g. page 5, lines 30 to 33; page 12, lines 19 to 27. In order to determine retention of enzyme functional activity during storage, a method as claimed would have to be performed, so its disclosure was implicit in the passage on page 10, lines 7 to 9.

- XII. The appellant requests that the decision under appeal be set aside and that a patent be granted on the basis of the claims of the main request filed with the statement of grounds of appeal or, alternatively, on the basis of the first or second auxiliary request filed with letter dated 12 May 2017.

Reasons for the Decision

1. The appeal is admissible.
2. In view of the fact that the present application has already been the subject of a previous appeal, the

board decided to grant the appellant's request for acceleration of proceedings.

3. Res judicata

3.1 The present application has been the basis for an earlier decision of a technical board of appeal: decision T 332/12 terminated the first appeal proceedings in examination, ruling that the claims of auxiliary request 9, filed with letter of 27 June 2013, fulfilled the requirements of Articles 123(2), 84 and 83 EPC. The present main request is identical to said auxiliary request 9. Hence, the conclusions reached in decision T 332/12 constitute *res judicata* for the main request. Concerning the auxiliary requests, the present board of appeal is also bound by the *ratio decidendi* of the previous decision, but only in so far as the facts are the same. As regards further amendments, these are to be fully examined as to their compliance with all requirements of the EPC.

4. Main request - Article 56 EPC

4.1 The application discloses stable compositions comprising both a nuclease (Exonuclease I: Exo I) and a phosphatase (shrimp alkaline phosphatase: SAP), which are to be used in DNA processing methods, e.g. prior to DNA cycle sequencing. Both D1 and D2 disclose methods of DNA sequencing which involve amplifying DNA by PCR and then processing of said amplified DNA by Exo I and an alkaline phosphatase such as SAP (see e.g. Example 6 of D1, in particular second paragraph of page 15; D2, page 858, last paragraph of middle column). Each of these two documents thus discloses compositions comprising Exo I and SAP (albeit in initially separate compositions) and hence can be considered the closest

prior art. The difference to the claimed subject-matter is that the specific composition as claimed, comprising both Exo I and SAP, at least 20% vol. of a stabiliser chosen from glycerol, ethylene glycol or glycine, and no amplified DNA, is not disclosed in either document.

4.2 According to the patent application, such a dual composition has the advantage over the single compositions of the prior art that multiple pipetting (with the experimental error potentially linked thereto) is avoided and the ratio between the two enzymes is held constant (page 3, second paragraph). Hence the technical problem can be formulated as the provision of an improved composition, namely one which is more convenient to handle and which allows for more experimental accuracy. The solution is a composition as claimed, comprising both Exo I and SAP, at least 20% by volume of a stabiliser and no amplified DNA, and the board is satisfied that the problem has been plausibly solved.

4.3 It remains to be examined whether the claimed solution involves an inventive step. The board considers that the skilled person, motivated to minimise errors potentially associated with multiple pipetting and to make handling more convenient in the DNA processing which is performed before cycle sequencing, would promptly consider preparing a dual composition comprising the two enzymes, in order to be able to administer them simultaneously in one step. There would be no reason to doubt that such a composition would be active, because both D1 and D2 show that the enzymes can be administered simultaneously in the same method step (see D1 and D2, supra) and nevertheless be functionally active. The further features of the composition, namely the absence of amplified DNA and

the presence of at least 20% by volume of a stabiliser, are also not considered inventive and are in fact already part of the prior art: the single compositions of the prior art do not contain amplified DNA (or non-amplified DNA, for that matter) and contain 50 vol.% glycerol, as disclosed in the application on page 2, second paragraph (for Exo I), and on page 3, first paragraph (for SAP). Hence the board cannot acknowledge an inventive step for the claimed subject-matter.

4.4 The appellant essentially argued that there was a prejudice in the prior art against providing the two enzymes in one composition because, as stated on page 3 of the application (lines 21 to 33), it was believed that they would be rendered inactive. As further evidence of the alleged prejudice the appellant referred to D4 and to expert declarations.

4.5 The board considers that there is no evidence on file for the existence of the alleged prejudice. The above-mentioned passage of the application states that "Historically, a stable composition comprising both enzymes in fixed proportion has not been commercially produced. It may have been thought that the $MgCl_2$ and $ZnCl_2$, both present in the commercial SAP storage buffer, were incompatible with the EDTA present in the commercial Exo I storage buffer." It thus appears that the alleged existence of a prejudice is based only on the fact that such a combined composition had not been produced in the prior art. However, the fact that a composition was not disclosed in the prior art cannot be taken as evidence that there was a prejudice against it in the prior art: otherwise, inventive step would have to be automatically acknowledged for any novel subject-matter.

4.6 The appellant argued that, as stated in the above-mentioned passage of the application, it would be expected that EDTA, present in the Exo I commercial composition, sequestered the Zn cations present in the SAP commercial composition, thereby rendering SAP inactive, because SAP required Zn^{2+} in order to retain full enzymatic activity. This is further explained in the declaration by one of the inventors, Moffett (D6, section 11). In this context, this latter declaration refers in section 13 to Olsen et al. (D4), which discloses that: "The shrimp alkaline phosphatase is completely inhibited by EDTA" (abstract 4). It should however be noted that D4 states in the same sentence that this inactivation is reversible, reactivation being achieved by addition of zinc: "..., but the activity can be restored to a large degree by zinc." Reactivation of the SAP enzyme is further discussed in other passages of D4, namely page 758, right column, last paragraph, and page 760, left column, last two lines, to right column, line 4. Hence, the board is not convinced that the skilled person would be taught away from combining the two compositions by fearing loss of SAP activity, because he would know that SAP's activity could if needed be restored by addition of zinc. In fact, the methods of D1 and D2 also use the two compositions together, in their commercial preparations, and, despite the presence of EDTA in the Exo I composition, report no lack of activity for any of the enzymes: that the enzymes are active is implicit from the fact that their omission in control experiments resulted in failure to provide "good sequence data" (D1, page 15, lines 19 to 21) and is explicitly stated in D2 (page 858, middle column, lines 12 to 13). Actually, both D1 and D2 teach that, in order to inactivate the enzymes once they are no longer needed, a further step is needed, namely heat

inactivation at 70°C: it can thus be concluded that without this inactivation step the two enzymes would still be active even after 15 minutes at 37°C. Accordingly, there would be no reason for a skilled person to doubt that both enzymes in the dual composition would remain active at least for 15 minutes at 37°C, which is the time that they are left to act in the methods of D1 and D2.

4.7 As to the appellant's argument that EDTA was considered necessary for Exo I activity in the prior art, the board notes that there is no evidence on file for such a statement. As discussed in Moffett's declaration (section 11), "EDTA is added to many enzyme preparations, particularly Exo I, as a preservative and stabilizer"; this is consistent with the application's teaching that chelating agents (such as EDTA) "are frequently added to protein solutions to sequester metal ions which if present can catalyze changes in amino acid side chain chemistry and under certain conditions cause breaks in the amino acid backbone of enzymes, thereby decreasing activity" (page 15, lines 10 to 15): it follows that, in the absence of such metal ions, no EDTA is necessary. The claimed composition neither excludes nor requires the presence of metal ions or EDTA, and hence such an argument does not apply.

4.8 The appellant further argued, based on another expert declaration (Horn's declaration, D7), that it was considered best practice at the priority date to keep purified enzymes separate from other enzymes, so as to enable the composition and storage conditions to be tailored to the specific enzyme and to avoid potential cross-reactions between different enzyme types. Moreover, there were concerns as regarded purity of the

available SAP enzyme, which would further teach away from combining it with another enzyme, due to fear of unpredictable interactions. Again the board considers that, while it might have been the normal practice at the priority date to use the two enzymes in separate compositions (because they were in any case only available separately), this does not mean that there was necessarily a prejudice against e.g. mixing them together in one sole stock solution before adding them to the reaction mixture. While the skilled person might indeed have considered the possibility of unwanted interactions or even inactivation of one or both enzymes resulting from the mixing of the two different buffer solutions, these fears could easily be allayed by routine testing of the stock solution and were in any case not justified in view of the results obtained in D1 and D2.

- 4.9 Moreover, the alleged prejudice would not necessarily apply to the subject-matter of claim 1, which is simply directed to a composition comprising the two enzymes (theoretically free of contaminants) and a buffer with at least 20 percent by volume of a stabiliser: there would be no reason to doubt that the enzymes would remain functionally active in such a buffer, or at least that their enzymatic activity could be restored by later addition of the necessary reagents (such as zinc in the case of SAP). In fact, the arguments concerning a prejudice are directed to the mixture of the two commercially available Exo I and SAP I stock solutions, rather than to the mixture of the two enzymes per se, which is however the subject of the claim. Therefore, the appellant's further arguments that the SAP reactivation disclosed in D4 only worked in the absence of EDTA or that it did not solve the problem of providing an easier-to-handle method (since

it required a further step of zinc addition) are likewise not relevant in the context of the claimed subject-matter, which does not require EDTA to be a component of the composition as claimed.

- 4.10 It is further noted that the present claim does not require that the enzymes are active for any minimum amount of time, i.e. that the composition as claimed is stable for a given period of time in terms of enzyme functional activity. Nor is there any data showing that enzyme stability in the combined preparation is identical or comparable to that of the single-enzyme preparations. In this context, while the board agrees with the appellant's argument that the claimed enzymes are active by definition, activity being an inherent property of the enzymes, it still notes that the same is not necessarily true as regards enzyme stability. Enzyme stability, i.e. the period of time during which enzyme activity is maintained, can in fact vary significantly with different storage conditions such as buffer composition and temperature, as is shown in the application in Table 1: of the five different compositions tested (all falling within the scope of the claim), compositions A and D present good enzyme stability (measured as activity half-life) at all tested temperatures, while the enzymes in compositions B and C are unstable at 4°C and 25°C (and even at -20°C for composition C). The post-published evidence submitted in D5 shows that the enzymes "retain well over 50% of their functional activity in composition C when stored for 24 hours at -20°C and at 4°C" (paragraph 6 of D5). However, apart from demonstrating functional activity after storage for 24 hours at 4°C, these results are not necessarily different from those presented in the application (Table 1), wherein it is shown that the activity half-

life of the enzymes in composition C at -20°C is less than 2 days.

4.11 The subject-matter of claim 1 of the main request hence lacks inventive step. The main request is not allowable for lack of compliance with Article 56 EPC.

5. First auxiliary request - Article 56 EPC

5.1 Claim 1 of this request differs from claim 1 of the main request in that the claimed composition further comprises a buffering agent, a reducing agent and a chelating agent.

5.2 According to the patent application: "The only necessary components of the invented composition are the enzymes, that is, the nuclease and the phosphatase"; other components are described as being "preferred but (...) optional" (page 12, lines 29 to 32). As regards the effect of the specific components in the claim, the application states the following: "Buffers or other agents may be added to control the pH of the solution thereby increasing the stability of the enzymes" (page 14, lines 21 to 23); "Reducing agents may be added to limit enzyme oxidation that might adversely affect stability of the enzymes" (page 14, lines 28 to 30); and "Chelating agents are frequently added to protein solutions to sequester metal ions which if present can catalyze changes in amino acid side chain chemistry and under certain conditions cause breaks in the amino acid backbone of enzymes, thereby decreasing activity" (page 15, lines 10 to 15). No surprising or unexpected effect is disclosed in the application in relation to the addition of any of these agents, which are in any case described as being only optional, nor is there any hint that their use

overcomes the alleged prejudice of the prior art, discussed above in relation to the main request. Hence, it is not apparent how this amendment might contribute to inventive step.

5.3 The same arguments as discussed above for the main request also apply to claim 1 of the first auxiliary request. As regards the prejudice concerning the presence of EDTA (a chelating agent) in the solution, again it is noted that addition of zinc would overcome the problem, as extensively discussed above. Contrary to the appellant's arguments, D4 does not show that complete removal of EDTA is necessary to reactivate SAP, since the passage cited by the appellant in this respect (page 756, right column, third full paragraph) in fact states that: "The final concentration of EDTA in the assay mixture was 1 μ M or less" (hence not completely absent). Present claim 1 does not require a minimum amount of chelating agent to be present.

5.4 The board thus comes to the conclusion that the subject-matter of claim 1 of the first auxiliary request lacks an inventive step. The first auxiliary request is therefore not allowable for lack of compliance with Article 56 EPC.

6. Second auxiliary request - Article 123(2) EPC

6.1 Claim 1 of the second auxiliary request is directed to a method of degrading nucleic acids in a sample, comprising the step of contacting the sample with a composition defined as in claim 1 of the first auxiliary request, and further defined by the feature "said composition (...) has been stored at -20°C for 24 hours prior to contacting the sample" (for the exact claim wording, see section IX).

- 6.2 The board fails to see where a basis can be found for the added feature. The passages indicated by the appellant are not considered to provide an adequate basis, for the reasons explained below.
- 6.3 Original claims 21 to 31 are directed to a method of degrading nucleic acids in a sample by contacting the sample with a composition comprising a nuclease and a phosphatase, but none of these claims further define the composition in terms of the specific enzymes used or of the further components as claimed or of the storage conditions prior to use. The Examples, on the other hand, do relate to storage of the compositions at different temperatures, including at -20°C , but do not disclose storage at any given temperature for 24 hours prior to contacting with the sample. The passages on page 4, line 10, and page 10, lines 7 to 9, refer to retention of at least 50% functional activity upon storage at 4°C for 24 hours: they do not relate to storage at -20°C , and they require a given functional activity which is not part of the claim.
- 6.4 The passage on page 12, lines 19 to 27, again refers to retention of a given percentage of functional activity for each enzyme "following storage of the composition for 24 hours, or 2, 3, 4, 5, 8, 10, 15, 20, 30, 40, 60, 80, 100, 120, 150, 180, 210, 240, 300, 360, 500, 1000, 1500, 2000 and/or 3000 days at 25°C , 20°C , 18°C , 10°C , 4°C , 0°C , -10°C , -20°C , -30°C , -40°C , -60°C , -80°C , -100°C , -150°C or -190°C ". As argued by the appellant, measurement of the functional activity involved performing a method as claimed after storage for a given period of time at a given temperature (page 5, lines 30 to 33). However, it also involved a further step, namely that of determining the functional

activity of the nuclease/phosphatase compositions, as disclosed e.g. on page 19, last paragraph, to page 20, line 2. Such a step is not present in claim 1, which is not directed to a method for determining enzyme functional activity but rather to a method for nucleic acid degradation using the enzymes. For such a method, there is no disclosure in the application as filed of a step of storage at any given temperature (let alone -20°C) for a given period of time (let alone 24 hours) prior to contacting with the sample containing the nucleic acids to be degraded.

6.5 Claim 1 thus contravenes the requirements of Article 123(2) EPC. Therefore, the second auxiliary request is not allowable.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairman:



M. Schalow

A. Lindner

Decision electronically authenticated