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**Datasheet for the decision  
of 21 February 2014**

**Case Number:** T 0878/10 - 3.3.08

**Application Number:** 98948328.4

**Publication Number:** 1015601

**IPC:** C12N15/53, C12N9/02, C12Q1/68

**Language of the proceedings:** EN

**Title of invention:**  
Thermostable Luciferases and Methods of Production

**Applicant:**  
Promega Corporation

**Headword:**  
Luciferase/PROMEGA

**Relevant legal provisions:**  
EPC Art. 83, 84  
RPBA Art. 13(1)

**Keyword:**  
Main request - requirements of the EPC (yes)

**Decisions cited:**

**Catchword:**



**Beschwerdekammern  
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Chambres de recours**

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Case Number: T 0878/10 - 3.3.08

**D E C I S I O N  
of Technical Board of Appeal 3.3.08  
of 21 February 2014**

**Appellant:** PROMEGA CORPORATION  
(Applicant) 2800 Words Hollow Road  
Madison, WI 53711-5399 (US)

**Representative:** Ogle, James  
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Patent- und Rechtsanwälte  
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**Decision under appeal:** **Decision of the Examining Division of the  
European Patent Office posted on 4 December 2009  
refusing European patent application No.  
98948328.4 pursuant to Article 97(2) EPC.**

**Composition of the Board:**

**Chairman:** M. Wieser  
**Members:** T. J. H. Mennessier  
C. Heath

## Summary of Facts and Submissions

- I. The applicant (appellant) lodged an appeal against the decision of the examining division dated 4 December 2009, whereby the European patent application number 98948328.4 with publication number 1 015 601 was refused. The application, entitled "*Thermostable luciferases and methods of production*", originated from the international application published as WO 99/14336.
- II. The decision was based on the main and four auxiliary requests filed with letter of 12 October 2009.
- III. The requests were refused for lack of clarity (Article 84 EPC; all requests), insufficiency of disclosure (Article 83 EPC; main request and auxiliary request 2) and added subject-matter (Article 123(2) EPC; auxiliary request 4).
- IV. Together with its statement setting out the grounds of appeal the appellant filed a new auxiliary request 1A and maintained its main request and auxiliary requests 1 to 4, which were considered by the examining division. Correction under Rule 139 EPC of Figure 19 was requested. Oral proceedings were requested as an auxiliary measure.
- V. On 6 September 2013, the board issued a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), which was sent together with the summons to oral proceedings and wherein the board expressed its provisional, not binding views.
- VI. On 5 February 2014, in reply to the board's communication, the appellant filed additional submissions which were accompanied by a new main

request and three new auxiliary requests to replace all previous requests. The request for correction under Rule 139 EPC of Figure 19 was withdrawn.

VII. With a letter dated 14 February 2014, the appellant re-filed its previous second and third auxiliary requests as its main and first auxiliary requests.

VIII. The main request consists of three claims which read:

"1. A luciferase consisting of the sequence:

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MEDKN ILYGPEPFYP LADGTAGEQM FYALSRYADI SGCIALTNAH
TKENVLYEEF LKLSCRLAES FKKYGLKQND TIAVCSENGL QFFLPPIIASL
YLGIIAAPVS DKYIERELIH SLGIVKPRII FCSKNTFQKV LNVKSKLKYV
ETIIILDLINE DLGGYQCLNN FISQNSDINL DVKKFKPYSF NRDDQVALV
MFSSGTTGVS KGVMLTHKNI VARFSLAKDP TFGNAINPTT AILTVIPFHH
GFGMMTTLGY FTCGFRVVLV HTFEEKLFLQ SLQDYKVEST LLVPTLMAFL
AKSALVEKYD LSHLKEIASG GAPLSKEIGE MVKKRFKLNK VRQGYGLTET
TSAVLITPNN DVRPGSTGKI VPFHAVKVVD PTTGKILGPN ETGELYFKGD
MIMKGYNNE EATKAIINKD GWLRSGDIAY YDNDGHFYIV DRLKSLIKYK
GYQVAPAEIE GILLQHPYIV DAGVTGIPDE AAGELPAAGV VVQTGKYLNE
QIVQNFVSSQ VSTAKWLRGG VKFLDEIPKG STGKIDRKV LRQMFEEKH
TNGL."
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"2. A polynucleotide which encodes a luciferase according to claim 1."

"3. A vector containing a polynucleotide according to claim 2."

IX. On 18 February 2014, the board informed the appellant that the oral proceedings scheduled for 6 March 2014 were cancelled.

X. The following documents are referred to in the present decision:

(D1) EP 0 524 448 A1 (published on 27 January 1993)

(D2) WO 95/25798 (published on 28 September 1995)

(D3) P. J. White et al., Biochem. J., Vol. 319, 1996, pages 343 to 350

(D4) E. I. Dementieva et al., Biochemistry (Moskow), Vol. 61, No. 1, 1996, pages 115 to 119

XI. The submissions made by the appellant, insofar as they are relevant to the present decision, may be summarised as follows:

#### Admissibility

The main request was filed in direct response to the board's communication pursuant to Article 15(1) RPBA. It overcame all the formal objections outlined by the board.

#### Article 123(2) EPC

Figure 19, line "49-7c6", page 1, lines 15 to 16 and page 5, lines 21 to 24 of the application provided a direct and unambiguous disclosure of the luciferase of claim 1, the polynucleotide of claim 2 and the vector of claim 3, respectively.

#### Article 84 EPC

Claim 1 recited all mutations of the specific mutant 49-7C6, in accordance with both Figures 19 and 27.

Article 83 EPC

The whole sequence of the mutant luciferase of claim 1 and a detailed mutagenesis protocol were disclosed in the application.

Article 54 EPC

None of the prior art documents disclosed the specific mutant luciferase of claim 1.

Article 56 EPC

The mutant luciferase of claim 1 solved the objective technical problem of providing an improved mutant beetle luciferase that was thermostable to such an extent that it retained at least 50% of its activity after at least two hours in an aqueous solution at 50 °C. The claimed subject-matter, having this unexpected technical effect, could not be derived in an obvious way from the disclosure in documents D2 and D3, either if taken into consideration alone or in combination.

- XII. The appellant requests that the decision under appeal be set aside and a patent be granted on the basis of claims 1 to 3 of the main request filed with letter of 14 February 2014 or, in the alternative, on the basis of the auxiliary request filed with the same letter.

## Reasons for the Decision

### Correction under Rule 139 EPC

1. In the examination proceedings (see point 2 of the communication of the examining division of 17 May 2005), the examining division has allowed appellant's request for a correction of Figures 27 to 31, 36 to 39, 41, 43, 45 and 46. The examining division found that this correction was in agreement with the requirements of Rule 88 EPC 1973 (Rule 139 EPC 2000). It agreed that the presence of two additional amino acid residues ((D) Aspartic acid and (P) Proline) in front of the initiation (M) Methionine residue in the polypeptide sequences represented in each of these figures was the result of an obvious error and that the correction requested, namely the deletion of the two amino acid residue, was obvious in the sense that it was immediately evident that nothing else would have been intended than what was offered as the correction.
2. The board sees no reason to deviate from the decision of the examining division.

### Main request

#### Admissibility

3. The main request has been originally submitted as auxiliary request 2 on 5 February 2014 (see Section VI *supra*), as direct reply to objections raised by the board in its communication pursuant to Article 15(1) RPBA. The amendments contained in the claims of this

request are straightforward, they do not raise new issues, do not contribute to the complexity of the appeal case and, accordingly, do not lead to a delay of the proceedings. Therefore, exercising the discretion conferred to it by Article 13(1) RPBA, the board admits the main request into the proceedings.

Article 123(2) EPC

4. Claim 1 is directed to a mutant luciferase which is derived from Ppe2 luciferase obtainable from *Photuris pennsylvanica*, known in the art.
5. Figure 19, line "46-7C6" taken together with page 5, lines 4 to 6 - stating that the mutations are aligned in Figure 19 (compare lines "Ppe2" and "49-7c6") - of WO 99/14336 (whose content is deemed to correspond to that of the application as filed) provides a direct and unambiguous disclosure of the luciferase according to claim 1. Furthermore, page 5, lines 21 to 24 provides an adequate support for both a polynucleotide according to claim 2 and a vector according to claim 3. Therefore, the main request meets the requirements of Article 123(2) EPC.

Article 84 EPC

6. The claims are clear, concise and supported by the description. Therefore, the main request meets the requirements of Article 84 EPC.

Article 83 EPC

7. The 46-7C6 mutant luciferase of claim 1 is defined by its amino acid sequence. The mutagenesis procedure, which was performed to obtain it, used, as a starting



material, a cDNA known from the prior art and is disclosed in detail in the experimental part of the application (see in particular from page 44, line 6 to page 47, line 2). The prior art cDNA, encoding the LucPpe2 luciferase derived from *Photuris pennsylvanica* (see Figure 45), was modified by substituting the Methionine residue at position 249 by a Threonine residue. The obtained, mutated luciferase, referred to in the application as LucPre[T249M] or Luc[T249M], was used as a reference for evaluating the thermostability of the further mutants derived therefrom (see Table 2 on page 71). This disclosure is sufficiently clear and complete to enable a skilled person to carry out the invention. Therefore, the requirements of Article 83 EPC are met.

Article 54 EPC

8. The prior art documents on file do not relate to a luciferase obtainable from *Photuris pennsylvanica* and, *a fortiori*, do not describe any mutant thereof (documents D1 to D4 indeed relate to either *Luciola cruciata/lateralis/mingrelica* luciferases or to *Photinus pyralis* luciferase). Therefore, the subject-matter of claims 1 to 3 is novel and meets the requirements of Article 54 EPC.

Article 56 EPC

9. The closest prior art is represented by document D3, which describes a thermostable mutant of luciferase from *Photinus pyralis* (see Figures 5 and 6 and the discussion regarding a comparison of the thermostability of the wild-type and mutant luciferase E354K on page 347). The technical problem to be solved in the light of document D3 is seen in the provision of

a further thermostable mutant luciferase. As a solution to this problem the application proposes the mutant luciferase of claim 1. In view of the experimental results presented in the application (see Table 2 on page 71), the board is convinced that the technical problem has indeed been solved.

10. Neither does any of the prior art documents on file (see D1 to D4) contain any hint to use as a starting material the luciferase obtainable from *Photuris pennsylvanica* nor is there, of course, any guidance to perform the particular mutations that lead to the achievement of the specific enzyme claimed, having the technical effect reported in Table 2 (see *supra*). Accordingly, the subject-matter of claims 1 to 3 cannot be derived in an obvious way from the prior art on file in an obvious way and, therefore, involves an inventive step in accordance with the requirements of Article 56 EPC.

## Order

### For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted to the examining division with the order to grant a patent on the basis of claims 1 to 3 of the main request filed with letter of 14 February 2014 and the description and drawings yet to be adapted thereto.

The Registrar:

The Chairman:



A. Wolinski

M. Wieser

Decision electronically authenticated