Datasheet for the decision
of 15 September 2010

Case Number: T 2355/08 - 3.3.08
Application Number: 04090321.3
Publication Number: 1627924
IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Method for the analysis of methylated DNA

Applicant:
Epigenomics AG

Headword:
Methylated DNA/EPigenomics

Relevant legal provisions:
EPC Art. 123(2), 84, 54, 56

Keyword:
"Claim request - added subject-matter (no), clarity (yes),
novelty (yes), inventive step (yes)"

Decisions cited:
T 1074/03

Catchword:
-
Case Number: T 2355/08 - 3.3.08

DECISION of the Technical Board of Appeal 3.3.08 of 15 September 2010

Appellant: Epigenomics AG
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Representative: Schubert, Klemens
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Decision under appeal: Decision of the Examining Division of the European Patent Office posted 10 June 2008 refusing European application No. 04090321.3 pursuant to Article 97(2) EPC.

Composition of the Board:
Chairman: L. Galligani
Members: P. Julià
J. Geschwind
Summary of Facts and Submissions

I. An appeal was lodged against the decision of the examining division dated 10 June 2008, whereby the European patent application No. 04 090 321.3, published as EP 1 627 924 (hereinafter referred to as "the application as filed"), was refused under Article 97(2) EPC.

II. In its decision, the examining division referred to its communication dated 27 November 2007 annexed to the summons to oral proceedings, wherein the applicant was informed that claims 1 to 14 as originally filed were considered not to fulfil the requirements of Articles 84, 54 and 56 EPC. The decision of the examining division was issued after the applicant withdrew its request for oral proceedings and requested a decision on the state of the file.

III. The applicant (appellant) filed a notice of appeal and a statement setting out its grounds of appeal which contained a new set of claims and experimental evidence (Enclosure I) in support of inventive step.

IV. The examining division did not rectify its decision and referred the appeal to the Board of Appeal (Article 109 EPC).

V. On 8 June 2010, the board issued the summons to oral proceedings to which a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) was attached. In that communication, the appellant was informed of the board's preliminary,
non-binding opinion on the issues to be discussed at the upcoming oral proceedings.

VI. On 11 August 2010, the appellant replied to the board's communication and filed a new claim request.

VII. At the oral proceedings, which took place on 15 September 2010, the appellant filed a new claim request in replacement of its previous request.

VIII. Claims 1 and 11 of the appellant's claim request read as follows:

"1. Method for the analysis of cytosine methylation in DNA, characterized in that the following steps are conducted:

a) a genomic DNA sample is chemically or enzymatically treated in such a way that all of the unmethylated cytosine bases are converted to uracil, while the 5-methylcytosine bases remain unchanged,

b) at least one oligonucleotide carrying a non-extendable 3' end is annealed to the converted DNA, wherein the non-extendable 3' terminus of the oligonucleotide is specific for the DNA of a defined methylation status,

c) the non-extendable 3' terminus of the oligonucleotide is only removed in case the oligonucleotide is bound without mismatch to the DNA with the methylation status to be detected, while hybridization to the background DNA takes only place under mismatch formation,
d) the unblocked oligonucleotide is extended, and

e) the methylation status is concluded from the absence or presence of an extended oligonucleotide product."

"11. A kit, which consists of oligonucleotides carrying a non-extendable 3' end, and a bisulfite reagent, wherein the non-extendable 3' terminus of the oligonucleotides is specific for a DNA of a defined methylation status."

Claims 2 to 10 were directed to particular embodiments of claim 1.

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

D1: US2004/0009515 (publication date: 15 January 2004);


X. The appellant's arguments, insofar as they are relevant to the present decision, can be summarized as follows:

Articles 123 and 84 EPC

Claim 1 was a combination of claims 1 and 2 as originally filed. The amended claims were directed to one of the two embodiments described in the application.
as filed and a basis was found inter alia in paragraphs [0007], [0015] and [0018] of the application as filed. All steps referring to a 3' blocked oligonucleotide were related to claim 1 by the feature of hybridizing without mismatch, i.e. the activation and removal of the non-extendable 3' terminus by pyrophosphorolysis only took place in case of a binding without mismatch.

**Article 54 EPC**

None of the cited prior art disclosed a kit comprising a bisulfite reagent and oligonucleotides that carried a non-extendable 3' end and were specific for a DNA of a defined methylation status.

**Article 56 EPC**

Document D3 disclosed the methylation-specific PCR (MSP) and represented the closest prior art. The technical problem to be solved was the provision of a method for methylation-specific amplification of DNA fragments with enhanced specificity as compared to MSP. The solution was provided in the application by applying oligonucleotides with one methylation-specific nucleotide that had a removable blocking function and was located at the 3' end of the oligonucleotide. Experimental evidence was on file showing that the claimed method provided a significant advantage over the MSP approach, namely an increased specificity.

The skilled person would not have chosen those oligonucleotides to increase the specificity of the MSP method. There were other common ways available to the skilled person, such as, for example, optimisation of
the MSP primer oligonucleotide design in order to cover more CpG positions and to have a greater number of nucleotides, or optimisation of the PCR reaction conditions (raise annealing temperature, higher concentration of MgCl₂, etc.). Indeed, the skilled person would not have expected those oligonucleotides to result in a higher specificity due to the reduced number of covered CpG nucleotides.

The claimed method was less cumbersome than other methods known in the prior art, such as the HeavyMethyl method that required the design and synthesis of two different kinds of oligonucleotides, namely unspecific primer oligonucleotides and methylation-specific blocking oligonucleotides. In contrast, the claimed method required only one oligonucleotide with a (non-extendable) blocked 3' end, wherein the removal of the blocking function required only the addition of PPi. The synthesis of oligonucleotides with non-extendable 3' ends did not require technical means going beyond the standard techniques used for oligonucleotide synthesis. The removal of the 3' blocking end by merely adding commercially available PPi did not require any additional working step. The claimed method was not obvious in view of the prior art, it did not represent a random modification of that prior art and it was not more cumbersome than other methods disclosed in the prior art, including the MSP method of document D3.

XI. The appellant (applicant) requested that the decision under appeal be set aside and a patent be granted on the basis of claims 1 to 11 filed during the oral proceedings.
Reasons for the Decision

Articles 123 and 84 EPC

1. No objections were raised by the examining division under Article 123(2) EPC, nor does the board see any reason for raising such an objection against the present claims. Basis for the claimed subject-matter is found in the application as filed and has been clearly indicated by the appellant (cf. point X supra).

2. The method of claim 1 for the analysis of cytosine methylation in a (genomic) DNA sample is characterized by the method steps (a) to (e), which are clearly defined in claim 1 and in the application as filed. The products required to perform these steps are also defined in claim 1 and well-known and available to the skilled person - as shown by the bibliographic references cited in the application as filed and the prior art documents on file, in particular, documents D1 to D3. Thus, the claimed subject-matter is considered to fulfil the requirements of Article 84 EPC.

Article 54 EPC

3. No novelty objections were raised by the examining division against the claimed method. The board does not see any reason to depart from this finding.

4. In the communication of the examining division referred to in the decision under appeal, Table 1 of document D2 and Table 6 of document D1 were cited as anticipating the claimed kit. Although both tables disclose one of
the two specific products characterizing the claimed kit, namely oligonucleotides carrying a non-extendable 3' end, wherein the non-extendable 3' terminus is specific for a DNA of a defined methylation status, there is no reference in these tables to a bisulfite reagent nor to the use of these oligonucleotides for preparing a kit with a bisulfite reagent. In fact, documents D1 and D2 disclose the method of pyrophosphorolysis activated polymerization (PAP) which is characterized inter alia by using those specific oligonucleotides. However, none of these two documents contains any reference to a bisulfite reagent. Therefore, the kit of claim 11, which contains these oligonucleotides and a bisulfite reagent, fulfils the requirements of Article 54 EPC.

**Article 56 EPC**

5. Document D3, which is considered to represent the closest prior art, discloses the methylation-specific PCR (MSP) assay. The MSP assay or method is used to characterize the methylation status of any block of CpG sites in a CpG island and it takes advantage of the known bisulfite-mediated chemical conversion of cytosine to uracil, followed by PCR using primers designated to distinguish methylated from unmethylated DNA, thereby discriminating between DNA modified by bisulfite and DNA that has not been modified. The MSP method comprises steps (a), (d) and (e) of the method of claim 1 but differs therefrom in that it does not contemplate the method steps (b) and (c) of claim 1.

6. Starting from this closest prior art, the technical problem to be solved is seen in the provision of an
improved MSP method, namely a MSP method with an increased specificity (cf. paragraph [0005] of the application as filed). The formulation of the technical problem does not make any contribution to the inventive step, because, as acknowledged in the case law, it is the normal task of the skilled person to be constantly occupied with the achievement of improvements of known products and/or methods (cf. "Case Law of the Boards of Appeal of the EPO", 6th edition 2010, I.D.8.10, page 205 and, inter alia, T 1074/03 of 8 May 2006, point 11 of the Reasons). The experimental data reported in the "Enclosure I", filed with appellant's grounds of appeal (cf. point X supra), show that the claimed method achieves the intended improvement by using oligonucleotides with a non-extendable 3' terminus such as those disclosed in the present application.

7. Although oligonucleotides carrying a non-extendable 3' terminus as those of the present application were known in the prior art, in particular from documents D1 and D2 which disclose the pyrophosphorolysis activated polymerization (PAP) method, there is no hint or reference in document D3 which directs the skilled person's attention to these oligonucleotides and to their use in the MSP method disclosed therein. Moreover, means and methods other than using oligonucleotides carrying a non-extendable 3' terminus were available and more straightforward for the skilled person when trying to increase the specificity of the MSP method disclosed in document D3, such as those referred to by the appellant (optimize length and/or design of the MSP oligonucleotide primers, PCR reaction conditions, etc) (cf. point X supra). In the board's view, the
combination of the teachings of document D3 with those of documents D1 or D2, i.e. to use the oligonucleotides carrying a non-extendable 3' terminus such as those disclosed in the PAP method of documents D1 or D2 in the MSP method described in document D3, could only be made with hindsight knowledge.

8. Contrary to the opinion expressed by the examining division in the communication referred to in the decision under appeal, the board considers that the distinguishing features of the claimed method (use of oligonucleotides carrying a non-extendable 3' terminus specific for the DNA of a defined methylation status and removal when bound without mismatch to the DNA with the methylation status to be detected) are not just random modifications of the disclosure of document D3. The experimental evidence "Enclosure I" filed with the appellant's grounds of appeal shows that the claimed method provides an advantageous effect over the MSP method of document D3, namely a significant decrease of unspecific (background DNA) amplification and thereby an increase in the specificity of the method.

9. Nor can the board agree with the contention of the examining division that the introduction of these distinguishing features renders the MSP method of document D3 more laborious and cumbersome. As convincingly argued by the appellant (cf. point X supra), the synthesis of oligonucleotides carrying a non-extendable 3' terminus such as those described in the present application does not go beyond standard techniques normally used for the synthesis of oligonucleotides such as those of document D3. The removal of the non-extendable 3' terminus when the
oligonucleotide is bound without mismatch to the DNA with the methylation status to be detected by using a DNA polymerase having pyrophosphorolysis activity (cf. paragraph [0019] of the application) cannot be seen as rendering the MSP method significantly more laborious and cumbersome. All the less so when other methods available in the art for analysing cytosine methylation in (genomic) DNA are considered, such as those cited in the present application and, in particular, the "HeavyMethyl" method which uses methylation-specific blockers and requires the synthesis of several oligonucleotide primers of different nature (methylation unspecific primer oligonucleotides and methylation specific blocking oligonucleotides) (cf. paragraph [0004] of the application).

10. In this context of known methods for determination of cytosine methylation in (genomic) DNA requiring the synthesis of several oligonucleotide primers, it is worth mentioning that document D1 refers to the known ligation-mediated PCR (LM-PCR) method. Document D1 discloses a modification of the LM-PCR method which comprises the use of the oligonucleotides carrying a non-extendable 3' terminus described for the PAP method in the PCR amplification of the LM-PCR method, i.e. after (genomic) DNA cleavage, first oligonucleotide primer extension and linker ligation, the modified LM-PCR method being called ligation-mediated PAP (LM-PAP) (cf. paragraphs [0048], [0049], [0177] and [0178] of document D1). Document D1 also contemplates the use of the LM-PAP method to examine and determine differential methylation and the level of methylation in (genomic) DNA (cf. paragraphs [0179] and [0180] of document D1). There is, however, no disclosure in this
document of the specific steps required for that
determination nor a suggestion or a hint to the
possible use of those oligonucleotides in other methods
known in the prior art for the analysis of cytosine
methylation. Thus, document D1 does not go beyond that
particular disclosure which, in the board's opinion,
when account is taken of the defining features of these
LM-PCR and LM-PAP methods, does not render the claimed
subject-matter on itself obvious.

11. It is noted that, apart from an objection of lack of
novelty (cf. point 4 supra), the examining division did
not specifically raise any objection under Article 56
EPC for the subject-matter directed to a kit.
Nevertheless, this subject-matter was explicitly
mentioned under the objection of lack of inventive step
raised against the claimed method for the analysis of
cytosine methylation in DNA. In the board's judgement,
also in view of the considerations made above in
respect of the method claimed, the combination of the
two components in a kit is not rendered obvious by any
of the documents on file alone or in combination.

12. Thus, the claimed subject-matter is considered to
fulfil 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 first instance with the order to grant a patent on the basis of claims 1 to 11 filed during the oral proceedings and a description to be adapted thereto.

The Registrar:     The Chairman:

A. Wolinski      L. Galligani