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Datasheet for the decision of 24 November 2020

Case Number: T 1214/15 - 3.3.08

Application Number: 10011728.2

Publication Number: 2298930

IPC: C12Q1/68

Language of the proceedings: EN

Title of invention:
Preparation of templates for nucleic acid sequencing

Patent Proprietor:
Illumina Cambridge Limited

Opponent:
Kilger, Christian

Headword:
Abasic site/ILLUMINA CAMBRIDGE

Relevant legal provisions:
EPC Art. 56
EPC R. 103(1)(a)
RPBA Art. 12(4)

Keyword:
Admission of new evidence - (no)
Main request - inventive step - (yes)
Reimbursement of the appeal fee - (no)

Decisions cited:
T 0530/95

Catchword:

Europäisches Patentamt
European Patent Office
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Beschwerdekammern

Boards of Appeal

Chambres de recours

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

Case Number: T 1214/15 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 24 November 2020

Appellant: (Patent Proprietor)	Illumina Cambridge Limited Illumina Centre 19 Granta Park, Great Abington Cambridge, Cambridgeshire CB21 6DF (GB)
Representative:	Pirson, Stefan M. Otto, Björn M. Hoefer & Partner Patentanwälte mbB Pilgersheimer Straße 20 81543 München (DE)
Respondent: (Opponent)	Kilger, Christian Wachtelstr. 4 14195 Berlin (DE)
Representative:	CH Kilger Anwaltspartnerschaft mbB Fasanenstrasse 29 10719 Berlin (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 14 April 2015 concerning maintenance of the European Patent No. 2298930 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. R. Vega Laso
	A. Bacchin

Summary of Facts and Submissions

I. European patent No. 2 298 930 with the title "Preparation of templates for nucleic acid sequencing" was granted from the European patent application No. 10011728.2 which is a divisional application of the European patent application No. 06755781.9.

II. The patent was opposed on the ground for opposition of Article 100(a) in conjunction with Articles 54 and 56 EPC.

III. In an interlocutory decision posted on 14 April 2015, an opposition division found that the subject-matter of the claims according to the main request (amended claims 1 to 15 filed under cover of a letter dated 12 March 2014) lacked an inventive step. However, the claims according to the auxiliary request 1 filed during the oral proceedings were considered to meet the requirements of the EPC. Hence, the opposition division found that the patent could be maintained on the basis of the auxiliary request 1.

IV. Claim 1 of the main request reads as follows:

"1. A method of generating a template for a nucleic acid sequencing reaction comprising,

(i) providing at least one double-stranded nucleic acid molecule, wherein both strands of the double-stranded nucleic acid molecule are attached to a solid support at the 5' end, wherein the double-stranded nucleic acid molecule comprises a target region to be sequenced and non-target sequences flanking the target region,

(ii) cleaving one strand of the double-stranded nucleic acid molecule at an abasic site, wherein the site for cleavage is positioned in the non-target sequence, and

(iii) subjecting the cleaved strand to denaturing conditions to remove the portion of the cleaved strand not attached to the solid support, thereby generating a single-stranded template for a nucleic acid sequencing reaction."

Dependent claims 2 to 9 are directed to various embodiments of the method of claim 1. Independent claim 10 is directed to a method of nucleic acid sequencing which comprises forming a template for nucleic acid sequencing using the method of any of claims 1 to 9. Dependent claims 11 to 15 are directed to embodiments of either the method of claim 1 or the sequencing method of claim 10.

V. Each the patent proprietor (appellant) and the opponent (the present respondent, in view of the later withdrawal of his appeal; see section VIII below) lodged an appeal against the interlocutory decision and submitted a statement setting out the grounds of appeal. The respondent submitted new evidence (documents (12) to (15)).

VI. Each party replied to the statement of grounds of appeal of the other party. Together with its reply, the appellant re-filed the sets of claims according to the main request and the auxiliary request 1 underlying the decision under appeal, and submitted three sets of claims as new auxiliary requests 2 to 4. The respondent filed additional evidence (document (16)).

VII. Pursuant to their respective subsidiary requests, the parties were summoned to oral proceedings. Due to the coronavirus pandemic, the oral proceedings had to be re-scheduled.

VIII. On 4 November 2020, the respondent withdrew the appeal, but remained party to the appeal proceedings as of right in accordance with Article 107, second sentence, EPC.

IX. In preparation of the oral proceedings, the board issued a communication in which it expressed a provisional opinion on some procedural and substantive issues, in particular issues relating to Article 56 EPC and Rule 103(1)(a) EPC.

X. Upon inquire by the registrar, the respondent informed the board that he would not attend the oral proceedings.

XI. Oral proceedings were held on 24 November 2020 in the presence of the appellant.

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

(1): WO 00/75374, published on 14 December 2000;

(3): WO 00/18957, published on 6 April 2000;

(9): WO 00/58329, published on 5 October 2000;

(12): European patent application No. 10011728.2, amended claims 1 to 15 filed on 13 September 2011;

(13): European patent application No. 10011728.2, amended claims 1 to 15 filed on 4 April 2012;

(14): European patent application No. 10011728.2, communication of the examining division pursuant to Article 94(3) EPC dated 2 November 2011;

(15): European patent application No. 10011728.2, applicant's submission dated 4 April 2012; and

(16): R.J. Roberts and K. Murray, 1976, Critical Reviews in Biochemistry, Vol. 4, No. 2, pages 123 to 164.

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

Main request - Article 56 EPC

The claimed subject-matter involved an inventive step. Document (3), which was considered to be the closest state of the art, disclosed products of solid-phase amplification reactions which displayed double-stranded "bridged" structures. Prior to sequencing, the "bridged" structures were linearized by heat denaturation or cleavage by restriction endonucleases.

As described in paragraphs [0009] and [0010] of the patent, the use of a restriction endonuclease for linearizing "bridged" structures was not suitable when sequencing templates with a target region of unknown sequence, because it could not be predicted whether the template comprised a recognition sequence for the endonuclease within the target region, which would result in the destruction of the template.

The method described in document (3) differed from the claimed method in the nature of the cleavage method and the location of the cleavage site outside of the target region. The technical effect associated with the distinguishing features of the claimed method was that the cleavage site could be controlled, because cleavage was effected at a residue which did not occur naturally in the DNA.

Starting from document (3), the problem to be solved was to provide a method for removing a double-stranded "bridged" structure without destroying the target sequence. The problem was solved by the claimed method which comprised cleaving one strand of the double-stranded nucleic acid molecule at an abasic site positioned in the non-target sequence.

Document (3) did not address the problem of target destruction by cleavage, nor provide a hint to the claimed solution. The skilled person would not be motivated by the teachings in document (3) to look for alternative methods for removing the "bridged" structures, and specifically not for methods which ensured that the target region to be sequenced was not destroyed.

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

Main request - Article 56 EPC

It was undisputed that document (3) represented the closest state of the art, and that the difference was that, in contrast to the enzymatic cleavage described in document (3), in the claimed method cleavage was at

an abasic site. As regarded the location of the cleavage site, document (3) described potential cleavage sites, either in the flanking regions or in a known part of the template. Since it was evident to a person skilled in the art that cleavage in the target region should be avoided, an inventive step could not be based on the cleavage site being located in the non-target region.

The sole difference between the claimed method and the method described in document (3) was the cleavage at an abasic site, instead of using a restriction endonuclease. There was no technical effect associated with that difference. Hence, the objective technical problem had to be formulated as the provision of an alternative cleavage method.

It was established case law that the knowledge of the skilled person was not limited to single documents, but included the common general knowledge. Restriction endonucleases had been known and used since the 1970's, and their properties were well known. Document (3) did not mention the problem that a restriction endonuclease might cleave within the target region, because it was a basic knowledge of the skilled person that restriction endonucleases were site-specific, and that restriction sites could occur in unknown sequences. Being aware of the drawbacks of using a restriction endonuclease, the skilled person would have taken the most straightforward approach and would have replaced the endonuclease recognition site by an abasic site as described in document (9). Thus, the claimed method was not inventive.

Request for reimbursement of the appeal fee

Reimbursement of the appeal fee was justified on the grounds of a procedural violation. The opposition division failed to discuss, either during the oral proceedings or in the decision under appeal, the line of argument on inventive step based on document (1) as the closest state of the art that had been put forward in the notice of opposition. This was a clear violation of the opponent's right to be heard and the requirement for a reasoned decision under Rule 111(2) EPC.

XV. The appellant requested that the decision under appeal be set aside, and that the European patent be maintained on the basis of claims 1 to 15 according to the main request filed on 12 March 2014 and re-submitted with the letter of 15 December 2015.

XVI. Before withdrawing his appeal, the respondent requested that the decision under appeal be set aside and the patent be revoked, and that the appeal fee be reimbursed. No further requests were put forward after withdrawal of the appeal.

Reasons for the Decision

Admission of new evidence filed in appeal proceedings

1. The evidence submitted by the respondent together with his statement of grounds of appeal (documents (12) to (15)) addresses the opposition division's findings on Article 84 EPC which concerned solely the claims of the auxiliary request 1 (see section 3.1.2.3 of the decision under appeal). Since this request was found to meet the requirements of the EPC and the patent

proprietor is the sole appellant, it follows from the principle of prohibition of *reformatio in peius* that neither the board nor the respondent can challenge the opposition division's decision that the patent could be maintained on the basis of the auxiliary request 1. Hence, documents (12) to (15) cannot be admitted and considered in appeal proceedings.

2. Document (16) was filed by the respondent as evidence for the common general knowledge of the skilled person in the framework of assessing inventive step. There is no doubt that this document could and should have been filed in opposition proceedings and the respondent did not put forward any reasons that justify the late filing of this evidence. Hence, the board, exercising its discretion under Article 12(4) of the Rules of Procedure of the Boards of Appeal (RPBA 2007), which is applicable in accordance with Article 25(2) RPBA 2020, decides not to admit this evidence.

Main request – Article 56 EPC

Claim 1

3. In the decision under appeal, the opposition division found that, starting from document (3) as the closest state of the art, the method of claim 1 was obvious in view of document (9) (see section 2.4.3 of the decision).

4. Document (3) describes methods for solid-phase amplification and sequencing of nucleic acids. The double-stranded nucleic acid templates described in document (3) comprise a target region to be sequenced and two non-target regions flanking the target region (designated Y and Z in document (3), see page 13,

lines 2 to 12), the complementary strands of the template being attached to a solid support at their 5' ends to form a "bridged" structure (see Figure 1(e)).

5. It is stated in document (3) that, once the double-stranded templates have been amplified to generate nucleic acid colonies (see passage from page 24, line 14 to page 26, line 20 and Figure 1), they are prepared for hybridization of the sequencing primer by treating them "*... so that all or part of the nucleic acid templates making up the colonies is present in a single stranded form*" (see page 28, lines 22 to 24). For this purpose, document (3) describes two types of treatment: heat denaturation and digestion with a restriction endonuclease (see page 28, lines 24 to 29). The restriction endonuclease "*... may be specific for either a sequence contained in the oligonucleotide sequences Y or Z or another sequence present in the template nucleic acid*" (see page 28, lines 29 to 32). After digestion the colonies are subjected to denaturing conditions (in particular, heat) to separate the two strands of the templates, and the colonies are washed to remove the non-immobilised strands, thus leaving attached single-stranded DNA in the colonies (see page 28, lines 32 to 36).

6. In the decision under appeal, the opposition division held that, since document (3) taught site-specific cleavage within a non-target region of the template nucleic acid, the sole difference between the method described in document (3) and the method of claim 1 was the cleavage means. While the method described in document (3) used a restriction endonuclease specific for a double-stranded form of a sequence in the template nucleic acid, claim 1 specified cleavage of

one strand of the double-stranded template at an abasic site (see step (ii)). The board shares these views.

7. However, the board does not share the opposition division's view that the problem to be solved is the provision of an **alternative** mode of site-specific cleavage. As the appellant argued, the technical effect linked to the cleavage at an abasic site is that the cleavage site can be controlled. Since this cleavage reaction requires a residue which does not occur naturally, the cleavage can occur only at the pre-determined cleavage site in the non-target sequence.

8. In contrast, a restriction endonuclease is specific for a particular (natural) nucleotide sequence which statistically may occur also in the target region. The restriction enzyme would then cleave not only at the pre-determined site in a non-target sequence, but also at one or more further sites within the target region to be sequenced. As a consequence, the target region would be shortened and, in the worst case, destroyed. As stated in the patent, this may be a particular problem for template nucleic acids of unknown sequence, because it cannot be predicted in advance whether a particular restriction enzyme will cut within the target region.

9. Since cleavage at an abasic site is - compared to the use of a restriction endonuclease - clearly advantageous, starting from document (3) as the closest state of the art, the problem to be solved has to be formulated as the provision of an **improved** method of generating a template for a nucleic acid sequencing reaction.

10. Document (3) does not mention any drawbacks in connection with the endonuclease treatment of the double-stranded template to obtain the single-stranded form. Thus, in the board's view it is doubtful whether the skilled person would be prompted to improve the method described in document (3) by specifically changing the cleavage means, as the opposition division stated in the decision under appeal.

11. The respondent argued that, at the priority date knowledge of restriction endonucleases and their properties and drawbacks would be common general knowledge of a person skilled in the art, a molecular biologist with experience in sequencing and template preparation. Accordingly, it would have been known to the person skilled in the art "*... that restriction endonucleases are site specific and that these restriction sites could occur in unknown sequences*" (see page 3, third paragraph of the respondent's reply to the appellant's statement of grounds of appeal).

12. If the respondent's argument is accepted, the question arises what course of action an average skilled person could and would take in order to solve the problem of uncontrolled cleavage within the target region. According to the jurisprudence of the Boards of Appeal, the notional skilled person in the field of biotechnology has a conservative attitude and does not possess any inventive capability. From him/her nothing more can be expected than the carrying out of experimental work by routine means within the framework of the normal practice of filling gaps in knowledge by applying existing knowledge (see, e.g., decision T 530/95 of 10 June 1997).

13. The respondent itself provided the answer to the question above, namely "*... the person skilled in the art knew several restriction enzymes and was able to select the most suitable enzyme for the task at hand, knowing that he should not selected*[sic] *restriction enzymes cutting in his target sequence*" (see page 3, third paragraph of the respondent's reply to the appellant's statement of grounds of appeal).

14. Hence, should the skilled person reading document (3) have realised that uncontrolled cleavage of the target region by the restriction endonuclease might be a problem, he/she would have used the routine means, i.e. suitable restriction endonucleases. Restriction enzymes that cleave at recognition sequences which rarely occur in natural sequences were well known in the art at the priority date.

15. The notional skilled person had no motivation to go a step further and modify the method of document (3) by using other cleavage means as described in document (9), in particular cleavage at an abasic site. In the board's view, the opposition division's finding that the choice of this particular site-specific cleavage method was only a "*... random selection among the many cleavage known in the art, and in particular in D9 ...*" is tainted with hindsight knowledge of the present invention.

16. For these reasons, the board concludes that, contrary to the opposition division's view, the method of claim 1 was not obvious to a person skilled in the art. Hence, an inventive step within the meaning of Article 56 EPC is to be acknowledged.

Claims 10 to 15

17. The arguments put forward by the respondent concerning claims 10 to 15 (see sections 8.3 and 8.3.1 of his statement of grounds of appeal) apply solely to the auxiliary request 1. As stated in paragraph 1 above, this request cannot be challenged. Thus, the respondent's arguments concerning these claims cannot be considered.

Request for reimbursement of the appeal fee

18. The respondent requested reimbursement of the appeal fee based on an alleged procedural violation (Rule 103(1)(a) EPC), arguing that the opposition division failed to discuss, either during the oral proceedings or in the decision under appeal, the line of argument on inventive step put forward in the notice of opposition, based on document (1) as the closest state of the art (see section XIV above).

19. Although the respondent has withdrawn his appeal, reimbursement may still be considered *ex officio* by the board in accordance with Article 114(1) EPC, if deemed equitable by reason of a substantial procedural violation.

20. In the communication of 2 October 2014 attached to the summons to oral proceedings in opposition proceedings, the opposition division took account of all the lines of argument on inventive step and, *inter alia*, expressed the view that, starting from document (1) as the closest state of the art and in view of document (9), the method of claim 1 according to the main request was not obvious to a person skilled in the art. Even though the respondent reiterated its line of

argument based on document (1) in his reply of 27 January 2015, it appears from the minutes of the oral proceedings before the opposition division posted on 10 April 2015 that the sole line of argument brought forward by the respondent and discussed at the oral proceedings was based on document (3) as the closest state of the art in combination with document (9). This has never been disputed.

21. In view of the circumstances of the present case, the board is not persuaded that the alleged procedural violation occurred. If the respondent wished his objection of lack of inventive step based on documents (1) and (9) to be discussed at the oral proceedings and decided upon by the opposition division, it was to him to put forward the objection at the oral proceedings, or at least refer to its written submissions in this respect, and indicate that they were maintained. Failing that, the opposition division had to assume that the line of argument based on document (1) as the closest state of the art had been abandoned.

22. In the absence of a substantial procedural violation, the respondent's request for reimbursement of the appeal fee cannot be granted.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent in amended form on the basis of claims 1 to 15 according to the main request filed on 12 March 2014 and, if required, a description to be adapted thereto.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated