

**Internal distribution code:**

- (A) [ - ] Publication in OJ  
(B) [ - ] To Chairmen and Members  
(C) [ - ] To Chairmen  
(D) [ X ] No distribution

**Datasheet for the decision  
of 22 November 2019**

**Case Number:** T 0845/16 - 3.3.08

**Application Number:** 07824052.0

**Publication Number:** 2084295

**IPC:** C12Q1/68

**Language of the proceedings:** EN

**Title of invention:**

Method for sequencing a polynucleotide template

**Patent Proprietor:**

Illumina Cambridge Limited

**Opponent:**

Kilger, Christian

**Headword:**

Sequencing method/ILLUMINA

**Relevant legal provisions:**

EPC Art. 56

EPC R. 115(2)

RPBA Art. 15(3)

**Keyword:**

Claims as granted - requirements of the EPC met - (yes)

**Decisions cited:**

**Catchword:**



**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 0845/16 - 3.3.08

**D E C I S I O N**  
**of Technical Board of Appeal 3.3.08**  
**of 22 November 2019**

**Appellant:** Kilger, Christian  
(Opponent) Wachtelstr. 4  
14195 Berlin (DE)

**Representative:** CH Kilger Anwaltspartnerschaft mbB  
Fasanenstrasse 29  
10719 Berlin (DE)

**Respondent:** Illumina Cambridge Limited  
(Patent Proprietor) Illumina Centre  
19 Granta Park, Great Abington  
Cambridge, Cambridgeshire CB21 6DF (GB)

**Representative:** Leißler-Gerstl, Gabriele  
Hoefer & Partner  
Patentanwälte mbB  
Pilgersheimer Strasse 20  
81543 München (DE)

**Decision under appeal:** **Decision of the Opposition Division of the  
European Patent Office posted on 8 February 2016  
rejecting the opposition filed against European  
patent No. 2084295 pursuant to Article 101(2)  
EPC.**

**Composition of the Board:**

**Chairman** B. Stolz  
**Members:** M. Montrone  
D. Rogers

## **Summary of Facts and Submissions**

- I. An appeal was lodged by the opponent (hereinafter the "appellant") against the decision of an opposition division to reject an opposition against the European patent No. 2 084 295, having the title "Method for sequencing a polynucleotide template".
- II. With its statement of grounds of appeal, the appellant submitted arguments as to why the subject-matter of the claims as granted contravened Article 56 EPC.
- III. In reply, the patent proprietor (hereinafter the "respondent") submitted arguments as to why the subject-matter of the claims as granted were based on an inventive step.
- IV. The parties were summoned to oral proceedings. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's provisional, non-binding opinion on some of the legal and substantive matters of the case. In reply thereto, the appellant announced that it would not be attending the oral proceedings without submitting substantive arguments in response to any of the issues raised in the board's communication.
- V. Oral proceedings before the board were held on 22 November 2019, in the absence of the appellant.
- VI. Claim 1 of the claims as granted reads:  
  
"1. A method for pairwise sequencing of first and second regions of a target double-stranded polynucleotide, wherein said first and second regions

are in the same target double-stranded polynucleotide, the method comprising:

(a) providing a solid support having immobilised thereon a plurality of double-stranded template polynucleotide each formed from complementary first and second template strands linked to the solid support at their 5' ends, and multiple copies of one or more 5'-end immobilised primers capable of hybridising to the 3' end of the first template strand;

(b) selectively removing the second template strands of the plurality of double-stranded template polynucleotides to allow hybridisation of the first template strands to the 5'-end immobilised primers;

(c) carrying out a first sequencing read to determine the sequence of a first region of the template polynucleotide by a sequencing-by-synthesis technique or by a sequencing-by ligation technique;

(d) carrying out an extension reaction to extend one or more of the immobilised primers to copy the first template strand to generate a second immobilised template strand;

(e) selectively removing the first template strands of the plurality of double-stranded template polynucleotides to allow hybridisation of a sequencing primer to the template strands generated in step (d);

(f) carrying out a second sequencing read to determine the sequence of a second region of the template polynucleotide by a sequencing by synthesis technique or by a sequencing-by ligation technique, wherein determining the sequences of the first and second

regions of the target polynucleotide achieves pairwise sequencing of said first and second regions of said target double-stranded polynucleotide."

Claims 2 to 13 define specific embodiments of the method of claim 1. Claim 14 defines a use of the method of claims 1 to 13.

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

D1: WO 2004/070005 (published 19 August 2004);

D2: WO 00/75374 (published 14 December 2000);

D9: WO 98/44151 (published 8 October 1998).

VIII. The appellant's submissions in writing, insofar as relevant to the present decision, may be summarised as follows:

*Articles 100(a) and 56 EPC - claim 1*

Document D1 represented the closest prior art for the method according to claim 1. The document disclosed a method for pairwise sequencing of double-stranded nucleic acids (see page 1, lines 6 to 13, page 3, lines 3 to 4, page 4, lines 23 to 26). The issue of whether or not the sense and antisense strands were present in single-stranded form only in document D1 was irrelevant, since during sequencing both strands had to be single-stranded for technical reasons. This was likewise specified in the method of claim 1. The method disclosed in document D1 differed from the claimed method in that it was silent on the features recited in steps (b), (d) and (e) of claim 1. The features of step

(a) in claim 1 were disclosed in Figure 1 of document D1. Although the features in step (a) required the presence of both strands of a template, it did not require their presence in double-stranded form. Since document D1 disclosed the sequencing of both strands of a double-stranded nucleic acid, the features of step (a) of claim 1 were directly and unambiguously disclosed.

Contrary to the opposition division's finding in the decision under appeal, a technical effect could not be ascribed to any of the distinguishing features referred to in steps (b), (d) and (e) of claim 1, since Figure 4 of the patent in suit did not show an improvement over the closest prior art method. Figures 4a, b and c rather disclosed the claimed method in general terms without providing any information or suggestion, let alone data, in support of an improvement, in particular not as regards the quality of sequencing results. The objective problem was therefore considered as the provision of an alternative sequencing method. Alternatively, if a technical effect might be ascribed to the distinguishing features, then it was the possibility to perform the claimed method on an array. In light of this effect, the technical problem was alternatively formulated as to provide a transfer of a sequencing method onto a clustered array.

As regards obviousness, the skilled person starting from the method of document D1 and faced with any of the two problems defined above, would directly arrive at the method of claim 1 by combining the teaching of document D1 with either that of document D2 or D9. The opposition division's finding was incorrect that the skilled person would not have looked at documents D2 or D9, which were rather concerned with methods of nucleic

acid amplification but not sequencing. In any case it was irrelevant in light of the alternative problem defined above. Facing this alternative problem, the skilled person would have considered documents that related to array preparations, for example, documents D2 and D9 which disclosed methods for amplification of nucleic acids on arrays (named bridge amplification).

Document D2 disclosed the missing steps of claim 1 (i.e. steps (b), (d) and (e)) in Figures 1 and 2. Although the document did not provide information on how to generate an array, the removal of either strand of the double-stranded nucleic acid was disclosed (see claim 1, Figures 1 and 2). Document D2 further disclosed that the amplification method was suitable for sequencing-by-synthesis reactions by reference to a labelling of nucleotides prior to their incorporation into a nucleic acid sequence (see page 5, second paragraph), since it was common general knowledge that both bridge amplification and sequencing reactions relied on immobilised primers.

Document D9 disclosed on page 5, last paragraph to page 6, first paragraph a method of nucleic acid amplification. It further mentioned that immobilised nucleic acids may be sequenced (see abstract, page 15, second paragraph). Immobilisation was performed in defined areas or colonies comprising a plurality of immobilised single or double-stranded nucleic acid molecules (see page 7, fourth paragraph, page 8, page 12, third paragraph). The formation of bridge structures and bridge amplification methods were mentioned in Figures 1 to 3 of document D9. The separation of double-strands into single-strands followed by sequencing was disclosed on page 53, third paragraph. Furthermore, the document mentioned pairwise



sequencing of first and second regions of target polynucleotides (see page 59, second paragraph). Thus the skilled person faced with the problem of transferring a method of sequencing onto an array would find all the information missing in documents D2 or D9 and would arrive in an obvious manner at the method of claim 1.

IX. The respondent's submissions, insofar as relevant to the present decision, may be summarised as follows:

*Articles 100(a) and 56 EPC - claim 1*

The claimed method differed from the method disclosed in the closest prior art document D1 in various aspects. The claimed method concerned the sequencing of immobilised double-stranded polynucleotide molecules, while (i) in document D1 immobilised single-stranded molecules were sequenced (see page 9, lines 24 to 27, page 14, lines 23 to 26, Figure 1); (ii) document D1 disclosed solid supports with immobilised single-stranded molecules only (see page 14, lines 23 to 24, Figure 1); (iii) document D1 was silent on 5'-end immobilised primers capable of hybridising to the 3'-end of the first template strand; (iv) document D1 was further silent on the features referred to in steps (b), (d) and (e) of claim 1.

Several technical effects were associated with these distinguishing features, including the formation of so-called bridge structures; an efficient regeneration of the removed strand; a stable fixation of the template at both ends on the solid support during sequencing; improved sequencing data read outs; and an efficient hybridisation of sequencing primers.

The technical problem to be solved was thus the provision of an improved and more efficient method of pairwise sequencing.

The method according to claim 1 was not an obvious solution to this problem, since document D1 contained no pointers to use immobilised double-stranded nucleic acids forming bridge structures, immobilised primers, the removal of complementary strands prior to sequencing, and the regeneration of the removed strands.

Pointers to these features were also not derivable from documents D2 or D9, since document D2 was silent on any sequencing method, while document D9 taught single-strand sequencing only. In fact document D9 did not mention regeneration of the second strand, including the sequencing of second regions on the regenerated and complementary strand.

X. The appellant requested in writing that the decision under appeal be set aside and that the patent be revoked.

XI. The respondent requested that the appeal be dismissed.

### **Reasons for the Decision**

1. The duly summoned appellant did not attend the oral proceedings, which in accordance with Rule 115(2) EPC and Article 15(3) RPBA took place in its absence.

*Claims as granted*

*Claim construction - claim 1*

2. The claimed method is directed to the pairwise sequencing of two regions located on the same double-stranded target polynucleotide. The technique of "pairwise" sequencing is likewise referred to as "paired-end" sequencing in the art (see paragraph [0007] in the patent).
- 2.1 In a first step, a solid support is provided on which the target polynucleotides are immobilised via their 5'-ends. The polynucleotides consist of first and second template strands that are complementary to each other. The solid support is further characterised in that also primers are immobilised thereon at their 5'-ends, which hybridise to the 3'-ends of the first template strands (step (a)).
- 2.2 Before sequencing starts (of a first region on a first template strand), the second template strands are selectively removed to allow the first strands to hybridise to the immobilised primers (step (b)). The primers provide a free 3'-OH group for a sequencing-by-synthesis or a sequencing-by-ligation reaction (step (c)).
- 2.3 Subsequent to the first sequencing reaction, the previously removed second strands are regenerated by extending the immobilised primers, wherein the first strands serve as templates for the second strands due to their complementary sequences (step (d)).
- 2.4 Before a second sequencing reaction starts (of a second region on a second template strand), the first strands

are selectively removed to allow a hybridisation between the ("newly generated") second template strands and sequencing primers (step (e)).

- 2.5 The second sequencing reaction completes the pairwise sequencing of first and second regions located on both strands of the same double-stranded polynucleotides (step (f)).
3. Since step (e) of claim 1 does not specify that the sequencing primers are immobilised, the claim encompasses the use of soluble and immobilised primers for performing the second sequencing reaction.
4. Double-stranded polynucleotides wherein both strands are immobilised at their 5'-ends to a solid support are characterised by so-called "bridge structures" (see Figures 1 to 5 of the patent), which are *inter alia* generated by "bridge amplification" reactions (see respondent's submission of 31 October 2016, page 3, third paragraph), for example, as described in documents D2 (see page 2, second paragraph), and D9 (see page 5, last paragraph to page 6, first paragraph, Figures 1 and 5).
5. It is a matter of dispute between the parties whether or not the feature "*double stranded template polynucleotide each formed from complementary first and second template strands linked to the solid support at their 5' ends*" referred to in step (a) of claim 1 encompasses two complementary single-stranded polynucleotides immobilised each at their 5'-ends to a solid support, or double-stranded polynucleotides of complementary strands only.

6. As set out above, the "*double stranded template polynucleotide*" recited in step (a) of claim 1 is immobilised on the solid support at the 5'-ends of both, its sense and anti-sense strands. The immobilised double-strands thus form a bridge structure, while complementary single-strands immobilised each at their 5'-ends have a linear structure (see e.g. Figure 1 of document D1).
  
7. In light of these considerations, the board concludes that the feature "*double stranded template polynucleotide each formed from complementary first and second template strands linked to the solid support at their 5' ends*" in step (a) of claim 1 imposes a structural limitation on the claimed method which excludes the immobilisation of two single-strands of complementary sequences on a solid support.

*Article 100(a) and 56 EPC - claim 1*

*Closest prior art*

8. It is common ground between the parties that document D1 represents the closest prior art. The document discloses *inter alia* a method for pairwise sequencing of complementary sense and antisense strands of immobilised nucleic acids (see e.g. page 1, lines 6 to 13, page 3, lines 3 and 4, page 4, lines 23 to 26, page 9, lines 22 and 23).
  
9. The appellant submitted that the method of claim 1 differed from that of document D1 only in the features recited in steps (b), (d) and (e), while the features of step (a) were disclosed on page 9, lines 25 to 28, page 13, lines 9 to 22 and Figure 1 of document D1.

10. As regards the features of step (a) in claim 1 and their disclosure in document D1, the board is not convinced by the appellant's arguments. While page 9, lines 22 and 23 in document D1 mentions that the template DNA may be "*single stranded or double stranded*", the whole passage relied on by the appellant is silent on how these template molecules are immobilised on a solid support, i.e. in single or double-stranded form, at both 5'-ends or solely at one end. The same applies to the passage on page 13, lines 9 to 22 of document D1. Furthermore, Figure 1 of document D1 discloses solely that single-stranded polynucleotides are immobilised on a solid support. Moreover, document D1 whenever referring to the sequencing of double-stranded polynucleotides uses immobilised complementary single-strands, in other words, denatured double-stranded polynucleotides (see e.g. page 3, line 4, page 18, line 28 to 32). Although Example 9 in document D1 is silent on how the double-stranded polynucleotides are immobilised on a solid support for sequencing, their linear structure shown in Figure 8 implies that the double-stranded structure has been denatured prior to immobilisation. Such a structure is different from the bridge structure implied by the immobilised polynucleotides referred to in step (a) of claim 1 (see above). Document D1 is also silent on 5'-end immobilised primers as likewise referred to in step (a) of claim 1.
11. Thus, the board agrees with the opposition division's finding in the decision under appeal, that the claimed method differs from document D1 in the features defined in steps (a), (b), (d) and (e) (see point 9.3.1 of the decision under appeal).

12. The definition of the technical problem to be solved is likewise disputed between the parties, since the appellant submitted that either no technical effects could be ascribed to the distinguishing features indicated above, or if at all, then that the claimed method offered the possibility to perform the sequencing reactions on an array. The respondent submitted that the bridge structure and, in particular, the fixation of double-stranded templates at their 5'-ends improved the quality of the sequence data as reported in paragraphs [0018], [0056], Figures 4a and 4c of the patent.
13. Although the board agrees with the appellant that the patent is silent on any comparative experimental data between the claimed method and the method of the closest prior art, the explicit statements about the read out quality disclosed in the paragraphs of the patent indicated above seem credible, and facts or evidence that may raise doubts that the improvements concerning the quality of the data read outs are not achieved by the claimed method have not been provided by the appellant. According to established case law, each of the parties to the proceedings bears the burden of proof for the facts it alleges. The opposition division was not convinced by the appellant's submission on this issue during the first instance proceedings (see point 9.3.1 of the decision under appeal), nor was the board, as indicated in its preliminary opinion (see point 19 of the communication dated 5 September 2019). Accordingly, the appellant has not discharged its burden of proof on this matter.
14. In light of these considerations, the board agrees with the opposition division that the technical problem is

to be defined as the provision of an improved method for pairwise sequencing.

15. The subject-matter of claim 1 is considered to solve the problem defined above in light of Figures 4a and 4c of the patent.

*Obviousness*

16. It remains to be assessed whether or not the skilled person starting from the closest prior art method and faced with the technical problem identified above would have arrived at the claimed method in an obvious manner.
17. As set out above, although document D1 mentions the sequencing of *inter alia* double-stranded nucleic acid molecules, the sequencing reaction is performed on immobilised single-strands only. Bridge structures of immobilised template polynucleotides and potential advantages resulting therefrom for sequencing are neither mentioned nor suggested. Thus, document D1 does not point to a sequencing method that is performed on double-stranded templates immobilised at their 5'-ends to a solid support. Consequently, the claimed method cannot be considered obvious for the skilled person based on the teaching of document D1 alone. This was also not argued by the appellant.
18. The appellant, however, asserted that the method of claim 1 was obvious for the skilled person starting from the method disclosed in document D1 combined with the teaching of documents D2 or D9.
19. As regards document D2, the appellant referred to page 5, second paragraph and Figures 1 and 2. Based on the



disclosure on page 5, it stated that nucleotides could be labelled prior to their incorporation into a sequence, which was an indication "*that the method is suitable for a sequencing by synthesis reactions*", and hence, disclosed directly and unambiguously "*bridge amplification and sequencing using immobilized primers (see above in combination with figure 1)*" (see page 15, second to sixth paragraph of the statement of grounds of appeal).

20. The board is not convinced by the appellant's submissions. As set out in the decision under appeal (see point 9.3.1, page 7, last paragraph), document D2 does not suggest that the method described therein for "*bridge*" amplification of nucleic acids may be used for sequencing too. The passage on page 5, second paragraph of document D2 mentions a method for **detecting** "*the presence or absence of one or more target molecules in a test sample*". There is, however, neither an explicit nor an implicit reference to a sequencing method of nucleic acids, such as sequencing-by-synthesis or sequencing-by-ligation reactions, since a method aiming at the detection of nucleic acids by labelled nucleotides is fundamentally different from any method that determines the sequence of a nucleic acid. Accordingly, since the passage concerns different subject-matter, the skilled person would not consider the teaching of document D2 when trying to solve the above mentioned technical problem.
  
21. Document D9 concerns a method of bridge amplification of immobilised polynucleotides in colonies, i.e. in defined areas (see page 5, last paragraph to page 6, first paragraph, Figures 1 and 5, claim 1). The document further discloses that the amplified single or double-stranded molecules can be sequenced. However,

whenever sequencing of double-stranded polynucleotides is referred to in document D9, the double-strands are denatured, and one of the strands is removed before a sequencing reaction is carried out (see abstract, page 15, second paragraph, page 49 to page 59, second paragraph, in particular page 51, third paragraph). Thus, document D9 is silent on sequencing regions located on both strands of a double-stranded target nucleic acid, in other words a double-strand sequencing, and does not point at potential advantages associated with the sequencing of double-stranded nucleic acids forming a bridge structure.

22. Therefore, since neither the teaching of document D1 alone nor the combination with the teaching of documents D2 or D9 gives the skilled person a hint to the sequencing of first and second regions on both strands of an immobilised polynucleotide in a bridge form, an inventive step must be acknowledged for the method according to claim 1 (Article 56 EPC).

**Order**

**For these reasons it is decided that:**

The appeal is dismissed.

The Registrar:

The Chairman:



L. Malécot-Grob

B. Stolz

Decision electronically authenticated