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Datasheet for the decision of 31 May 2021

Case Number: T 2379/17 - 3.3.08

Application Number: 10000700.4

Publication Number: 2189540

IPC: C12Q1/68

Language of the proceedings: EN

Title of invention:
Methods for sequencing a polynucleotide template

Patent Proprietor:
Illumina Cambridge Limited

Opponent:
Kilger, Christian

Headword:
Pairwise sequencing of a polynucleotide template/ILLUMINA CAMBRIDGE LTD

Relevant legal provisions:
EPC Art. 100(a), 56, 100(b), 100(c)
RPBA 2020 Art. 13(2)

Keyword:
"Main Request - requirements of the EPC met (yes)"

Decisions cited:

Catchword:

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Case Number: T 2379/17 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 31 May 2021

Appellant: (Opponent)	Kilger, Christian Wachtelstr. 4 14195 Berlin (DE)
Representative:	CH Kilger Anwaltspartnerschaft mbB Fasanenstraße 29 10719 Berlin (DE)
Respondent: (Patent Proprietor)	Illumina Cambridge Limited Illumina Centre 19 Granta Park, Great Abington Cambridge, Cambridgeshire CB21 6DF (GB)
Representative:	Pirson, Stefan 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 10 October 2017 rejecting the opposition filed against European patent No. 2189540 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	R. Winkelhofer

Summary of Facts and Submissions

I. European patent No. 2 189 540, based on European patent application No. 10 000 700.4 - a divisional application of the earlier European patent application No 06765039.0 (published as International patent application WO 2007/010263; hereinafter "the parent application") - was opposed on the grounds of Articles 100(a) in conjunction with Article 56 EPC, and Article 100(b) and (c) EPC. An opposition division rejected the opposition.

II. The opponent (appellant) lodged an appeal against the decision of the opposition division. Together with their statement of grounds of appeal, they submitted new document D19.

III. In reply to the appeal, the proprietor (respondent) requested that the appeal be dismissed and submitted an auxiliary request 1.

IV. The parties were summoned to oral proceedings. In a communication pursuant to Article 17(1) RPBA 2020, the parties were informed of the board's provisional opinion, inter alia on issues concerning Articles 100(a) to 100(c), 76(1), 83 and 56 EPC.

V. In a submission dated 27 May 2021 the appellant requested that the oral proceedings be postponed or held by videoconference.

VI. Oral proceedings were held in Haar on 31 May 2021 in the presence of the respondent.

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

"1. A method for pairwise sequencing of first and second regions on complementary strands of a double-stranded polynucleotide template, the method comprising:

(a) providing a solid support comprising a polynucleotide template:
(b) carrying out a first sequencing-by-synthesis reaction on the solid support to determine the sequence of a first region of the first template strand,
(c) extending the strand complementary to the first template strand by sequential addition of further nucleotides, which do not contain any labels required for sequencing, to the last nucleotide added in step (b), thereby defining a second region complementary to the first template strand;
(d) ligating the 5' end of a self-complementary hairpin linker polynucleotide comprising a free 3’ hydroxyl group to the free 3' end of the extended strand; and
(e) carrying out a second sequencing reaction to determine the sequence of the second region complementary to the first template strand."

Dependent claims 2 to 10 define specific embodiments of claim 1.

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

D1: WO2004/07005 (published 19 August 2004);

D3: WO02/061127 (published 8 August 2002);

D19 WO97/04131 (published 6 February 1997).

IX. The submissions made by the **appellant**, insofar as relevant to the present decision, are summarized as follows:

In a submission dated 27 May 2021 appellant requested that the oral proceedings be postponed or held by videoconference.

Main request (claims as granted)
Article 76(1) EPC

First, the method of claim 1 comprised a step (a) of *"providing a solid support comprising a polynucleotide template"*, whilst the parent application disclosed a step of "providing a polynucleotide template having a first free 3' hydroxyl group which is positioned to initiate sequencing of a first region for sequencing determination on a first template strand".

The solid support of claim 1 was defined to "comprise" a polynucleotide template, while the parent application specified that the solid support was either "linked" to the polynucleotide template or to the linker polynucleotide (see claim 3). The parent application disclosed nowhere a generic embodiment where a solid support had to comprise only a polynucleotide template. Claim 1 extended to embodiments where a molecule comprising a polynucleotide template was linked to a solid support but by other means.

Secondly, the polynucleotide template was not required to "have a first free 3’ hydroxyl group which was positioned to initiate sequencing of a first region for

sequencing determination on a first template strand", in contrast to claim 1 of the parent application. It covered embodiments where the polynucleotide sequence was attached either by its 3' or by its 5' end to the solid support or wherein its first free 3'-OH was deleted. These embodiments required the use of a primer annealing to the polynucleotide template to provide the missing free 3'-OH required for sequencing.

Since the embodiments without a first free 3'-OH required the use of a primer annealing to the polynucleotide template (Fig.1A,(1)), but the polynucleotide template strand itself (Fig.1A,(3)) was not linked to the solid support (alternative 1), the primer had to be the element linked to the solid support (alternative 2) (see page 13, line 22 to page 14, line 7). For the embodiments of alternative 1, when the template strand was linked at its 3' end to the solid support, the first free 3'-OH was consumed and could not act as a primer for the template as specified in original claim 1 a). There was no requirement that a first free 3'-OH had to exist elsewhere on the molecule in these embodiments. On page 13, lines 11 ff., the 3' end of the polynucleotide template was ligated to a primer, which acted as a primer on the polynucleotide template and annealed on said template sequence with a first free 3'-OH. This limitation was lost in step a) of claim 1.

Thirdly, the method of claim 1 comprised a step (c) of "*extending the strand complementary to the first template strand* ***by sequential addition of further nucleotides, which do not contain any labels required for sequencing,*** *to the last nucleotide added in step (b),* ***thereby defining a second region*** *complementary to the first template strand*", whilst there was no direct

and unambiguous disclosure of such a requirement in the parent application.

There was no direct and unambiguous basis for this combination of features neither on page 7, lines 4 to 11, page 13, lines 6 to 9, Figures 3 to 6, nor on page 16, lines 14 ff. or 20 to 27 of the parent application.

Claim 8 of the parent application did not specify that the added nucleotides lacked any label required for sequencing.

Sufficiency of disclosure (Article 100(b) EPC)

The method of claim 1 was not sufficiently disclosed. It did not define in what orientation the template was attached to the solid support. In case the 3' end of the polynucleotide template was used to covalently link said template to the solid support, then there was no free 3'-OH group available for carrying out the sequencing-by-synthesis reaction of step (b). Without the use of a primer and of a free 3'-OH group, there were no instructions where and how the first nucleotide could hybridize to in the sequencing reaction. Claim 1 lacked technical details as to how the sequencing reaction was carried out.

If several primers were added to the polynucleotide template, immobilized or not, this could have generated partial double-stranded templates that prevented the extension reaction of step (c) due to their hybridization location. In the alternative, if an internal primer was used in the sequencing reaction, the sequencing of the whole strand, depending on the length of the polynucleotide template strand, was

simply unfeasible which prevented the subsequent step of the method from being carried out as well.

Nor was it disclosed in a manner sufficiently clear how the detection of incorporated nucleotides was performed at the rate of processivity of a polymerase and how the incorporated nucleotides were detected, nor whether and how the labels of the nucleotides were removed.
There was no teaching how the number of incorporated nucleotides had to be determined and how many nucleotides were added as defined in step c). The extension reaction could cover the full length template strand resulting in a double-stranded nucleotide. This fully extended full length template strand could not be sequenced as required in step (e) of claim 1, unless the first polynucleotide template strand was removed. However, this essential step was missing in claim 1. Since the extended second template region did not exclude very large polynucleotide template regions, their sequencing, as required in step (e) was, depending on their length, technically impossible.

Several functional features of claim 1 could not be realized without inventive efforts. The "second region" had an undefined length, which could be so large that its nucleotide sequence could not be determined anymore, and furthermore was defined to be separated by at least one nucleotide from the first region (see patent [0016] and figures 1 A, B, C and 2 A, B, C). The second region started with the first nucleotide added to the last nucleotide added in step b), defining the first region. This was however in contradiction to the definition provided in the description.

The addition of nucleotides for the second sequencing step of the method of claim 1 e) was not possible at the rate and efficiency required for sequencing.

Inventive step (Article 56 EPC)

Document D1 represented the closest prior art. It related to methods of sequencing both the sense and antisense strands of DNA through the use of blocked and unblocked sequencing primers. The double-stranded DNA was however comprised of two single-stranded DNAs referred to as a first single-stranded DNA and a second single-stranded DNA (see page 7, lines 7 to 13). Two primers (one blocked and one unblocked) were used to sequence both ends of a double-stranded nucleic acid (see page 3, lines 13 to 14). Since the two capture primers could anneal to two separate but complementary strands of the DNA, the capture beads contained both strands of the template DNA (see page 14, lines 20 to 26).

The difference between the method in document D1 and claim 1, was that claim 1 used a double-stranded template and a hairpin linker instead of a primer. The technical effect underlying this difference was that it reduced the number of hybridization steps, which increased the ligation efficiency of the sequencing method.

Thus, the technical problem was defined as the provision of an improved method for sequencing a first region of one strand of a polynucleotide and a second region of the complementary strand.

The solution was the method of claim 1.

Document D3 disclosed the sequencing of polynucleotide templates forming part of an array. The immobilization of the templates on a solid support was achieved by covalent or non-covalent binding of the templates at their 5' or 3' end, or via a linker, which could be a hairpin loop structure (see page 6, lines 11 to 29). The determination of the sequence was carried out using a primer by the incorporation of a labelled base at pre-determined positions followed by non-labelled bases (page 6, line 29; page 9, lines 20 to 29).

Thus, document D3 taught the immobilization of the hairpin to a solid support to provide an array and the sequencing of a polynucleotide bound thereto.

Document D1 provided an incentive to look for an improved method as it explicitly mentioned that the sequencing based on chemical cleavage was difficult to automate. Other sequencing methods were labor intensive due to the need to perform a hybridization step for every sequencing effort (see page 2, lines 18 to 21).

Document D1 provided also an incentive to improve the sequencing protocol and thus to combine document D1 with document D3 using hairpin linkers, as immobilized primers linked to hairpins increased the ligation efficiency in the sequencing method. Moreover, it led to identical sequencing reaction products after the first sequencing reaction (i.e. a sequencing product obtained from a first region of a template). Thus, the skilled person realised that the methods could be exchanged without the need for blocked primers.

A "pointer" was actually not required for concluding that a subject matter or the combination of two documents was obvious. In accordance with the case law

of the Boards of Appeal, a course of action could be considered obvious within the meaning of Article 56 EPC if the skilled person would carried it out in expectation of some improvement or advantage (T 2/83, OJ 1984, 265). In other words, obviousness was not only at hand when the results were clearly predictable but also when there was a reasonable expectation of success. The skilled person would have tried the solution with a reasonable expectation of success, as it involved only a change in steps and the application of a previously well known hairpin linker, allowing more sequencing data or a better method to be obtained. In the worst case it was obvious to try it out.

Alternatively, document D19 disclosed a method of amplifying a polynucleotide hairpin using a single primer. The method could be used to amplify double-stranded polynucleotides and to detect hairpin, double-stranded and single-stranded polynucleotides (see abstract; page 1, lines 23 to 25). This method was useful for the amplification and sequencing of unknown regions of DNA flanking regions of known sequence (see page 3, lines 1 to 3, Figure 5, page 3, lines 23 to 28). It required only one single primer. The ability to bind a primer to the hairpin adapter allowed the determination of the sequence adjacent to the hairpin adapter following hairpin amplification (see page 19, lines 11 to 15). The use of single-stranded DNA or RNA samples was disclosed on page 20, lines 9 to 11).

The difference between the method in document D19 and claim 1 was that a hairpin linker was linked to a double-stranded DNA instead of a single-stranded DNA and that it was not immobilized on a solid support. Thus, faced with the technical problem of amplifying and sequencing an unknown DNA sequence, the skilled

person would have obviously combined the teaching of document D1 and D19 to arrive at the subject-matter of claim 1.

Document D19 disclosed that the binding of a hairpin adapter to an unknown double-stranded DNA sequence would result in amplifying (and therefore sequencing) the unknown template strand under amplification conditions using only one primer when the strand following the loop of the hairpin was extended (see p. 3, lines 26 to 28; Fig.5). The ability to bind a primer to the hairpin adapter allowed the determination of the sequence adjacent to the hairpin adapter following hairpin amplification. Thus, the skilled person realized that in effect no primer was necessary and would have arrived at the subject-matter of claim 1 without inventive activity.

X. The submissions made by the **respondent**, insofar as relevant to the present decision, are summarized as follows:

Main Request
Article 76(1) EPC

The method of claim 1 step (a) described unambiguously that the polynucleotide template strands, in general, and not only a polynucleotide template having a free 3'-OH group, could be provided on a solid support (see parent application page 13, line 22 to page 14, line 7; page 16, lines 17 to 20; page 13, lines 11 to 19, and Figure 1).

The method of claim 1 step (b) used a sequencing-by-synthesis (SBS) method which required a free 3 -OH group to synthesize a polynucleotide complementary to

the template strand (see parent application on page 8, lines 11 to 20). Several options for providing this free 3 -OH group were disclosed, for example via a hairpin linker or a hybridizing primer (see page 13, lines 14 to 19 and 26 to 30).

The method of claim 1 step (c) described first and second regions for sequence determination of a double-stranded template (see parent application on page 7, lines 4 to 11; page 13, lines 6 to 9; Figures 3 to 6; page 16, line 14 and 20 to 27 and Fig. 3). Hence after the first sequencing reaction, the strand complementary to the first template strand was extended as defined in step (c), and thereby defined a second region to be sequenced in step (e) as described on page 10, line 29 to page 11, line 26, and on page 17, lines 19 to 22.

Sufficiency of disclosure (Article 100(b) EPC)

The skilled person was familiar with Sequencing-By-Synthesis (SBS) techniques, and their basic requirements, such as how to detect incorporated nucleotides, how to remove labels and how to determine the number of added nucleotides etc. He/she knew which orientation of the templates immobilized on the solid support was suitable for the method as granted and that an initiation point providing a free 3'-OH group, either by a primer or by the template strand itself was needed. The skilled person was provided sufficient support, in the light of its common general knowledge, how to carry out the claimed invention. The skilled person, with a mind willing to understand, would not have interpreted the claim in an unreasonable way to arrive at an allegedly non-working embodiment. It would never have carried out an SBS technique without providing all its elements, for example an initiation

point for the extension of the nucleotide strand etc. He would have used either a primer or a template strand with an adequate initiation point (free 3'-OH group) as disclosed in the paragraph bridging page 13 and 14.

The preamble of claim 1 stated that first and second regions on complementary strands of a double-stranded polynucleotide template were pairwise sequenced, that a first SBS reaction was carried out to determine the sequence of a first region, followed by an extension of the strand complementary to the first strand and that the template had a first and a second region, which were unambiguous terms in the context of pairwise sequencing. Claim 1 would not have been interpreted by the skilled person in an unreasonable way, wherein the first region encompassed the whole template strand, allegedly making it impossible to carry out the extension step c).

Embodiments that required displacement of the first template strand, once the double-stranded template was generated in steps c) and d), were achieved by the use of a strand-displacing polymerase or by nicking the template strand, enabling a sequencing of the second region as defined in step e) (see page 18 and Fig. 3D and E).

In the absence of serious doubts, substantiated by verifiable facts, appellant failed to establish that a skilled person could not, due to experimental uncertainties or an undue burden, perform the claimed methods in accordance with Article 83 EPC.

Inventive step (Article 56 EPC)

Document D1 was considered to represent the closest prior art. It disclosed an emulsion PCR method on beads which was only concerned with single-stranded templates.

The method of document D1 differed from the method of claim 1 in that it was a method for pairwise sequencing of first and second regions on complementary strands of a double-stranded polynucleotide template.

The method in document D1 disclosed an immobilization step of a single-stranded polynucleotide on a solid support. This was done for both single strands of a double-stranded polynucleotide. The templates to be sequenced were single-stranded or denatured double-stranded (see on page 3, lines 3 to 4; on page 7, lines 9 to 13; and on page 14, lines 23 to 26). There was no disclosure in document D1 of a method for pairwise sequencing of first and second regions on complementary strands of a double-stranded polynucleotide template as defined in claim 1. Steps c), d) and e) of the method of claim 1 were all absent from the method of document D1 and did not inherently form part of it.

The benefits and effect of pairwise sequencing were that two sequence "reads" from two places on a single polynucleotide template occurred on a single template, known to be very close one another in the genome (see patent paragraph bridging pages 2 and 3).

The hairpin linker in step d), providing the initiation point for the second sequencing reaction, rendered the use of a second primer which required fore-knowledge of a part of the sequence to be determined and the necessary hybridisation step in solution for a second sequencing reaction altogether dispensable (see page

12, 2nd paragraph of the description as filed). A primer hybridisation step would not be required at all if the first sequencing reaction was started from a free 3'-OH group on the template itself (see description as filed page 17, lines 22 to 28).

The problem to be solved was therefore seen as to provide an improved sequencing method providing more information which was suitable to determine the sequence of unknown templates, and which was easier to handle than the methods of the prior art.

The solution was the method of claim 1.

Document D19 disclosed a method of exponentially amplifying a polynucleotide hairpin using a single primer. The main purpose of the amplification was either the provision of enough material to be detected or enough material to be sequenced subsequently and could not be equated, let alone reduced, to a sequencing step. For amplification of the polynucleotide hairpin it was first ligated to double-stranded templates to be amplified and second was not immobilized on a solid support. It was carried out in solution.

Thus, the skilled person faced with the technical problem identified above had neither a hint nor a motivation to modify the method of document D1 concerned with single-stranded templates by combining it with either document D3 or D19.

XI. The appellant requested that the decision under appeal be set aside and the patent be revoked. They further request reimbursement of the appeal fee.

XII. The respondent requested that the appeal be dismissed, or alternatively, that the decision under appeal be set aside and the patent be maintained upon the basis of auxiliary request 1.

Reasons for the Decision

Format and date of oral proceedings

1. On 11 August 2020, the parties were summoned to oral proceedings on 31 May 2021 in Haar (Munich).

2. In a submission dated 27 May 2021 the appellant requested that the oral proceedings be postponed or held by videoconference. In view of "the President's decision and the present CORONA situation", and that they would need to fly to Munich, the appellant "expected" the board to issue a communication to this effect.

   The respondent reacted on the same day stating that they did not consent to changing the format of the proceedings to a videoconference.

3. According to Article 15(2) RPBA 2020, a change of date for oral proceedings may exceptionally be allowed at the board's discretion on receipt of a written and reasoned request made as far as possible in advance of the appointed date.

4. The appellant has submitted their request for either postponing or holding the oral proceedings by videoconference basically three days ahead of the oral proceedings, with a weekend in between.

4.1 They provided no specific reasons, related to the COVID-19 pandemic or not, as to why they would have been prevented from attending the oral proceedings in person. On the day of the oral proceedings, and also the week before, there were no travel restrictions within Germany for business purposes under the applicable Covid-19 rules. Having their offices in Berlin with convenient train connections with Munich, the appellant's representatives were therefore not prevented from attending proceedings in Haar.

5. When talking about "the President's decision ...", the appellant is not entirely clear to which President the appellant is referring: the President of the Board's of Appeal or the President of the EPO. However, neither of the two Presidents has issued a communication supporting the appellant's expectation.

5.1 The President of the Boards of Appeal, in a communication dated 15 December 2020 entitled "Oral proceedings before the Boards of Appeal – continuation of the measures adopted due to the coronavirus (COVID-19) pandemic and revised practice on oral proceedings by VICO", informed the public that the Boards of Appeal had reassessed some of their measures for the arrangement and conduct of oral proceedings.

The second paragraph of this communication underlined that these measures differed from those decided by the President of the European Patent Office for oral proceedings before examining and opposition divisions.

The third paragraph of this communication mentioned explicitly that if a party cannot attend oral proceedings for which they have been summoned, they

would have to request a change of date pursuant to Article 15(2) RPBA 2020.

The last paragraph of this communication underlined that all parties to scheduled oral proceedings were advised to check the web section of the Boards of Appeal regularly for updated information.

6. The second paragraph of the above communication referred also, by means of a hyperlink, to the "Information on oral proceedings before examination and opposition divisions as from 4 January 2021" dated 10 October 2020, which referred to Article 2 of the decision of the President of the European Patent Office dated 10 November 2020. This latter information mentions explicitly in its last paragraph that "... these measures differ from those decided by the President of the Boards of Appeal for oral proceedings before the Boards of Appeal."

7. The appellant's expectation to receive an invitation from the board to participate by video conference was therefore without basis.

8. Ultimately, while it is possible to arrange oral proceedings in a so called mixed mode with one party attending on site and another by video conference, such arrangements require more than one effective workday's notice for technical reasons.

9. In view of the circumstances of the present case, the appellant's requests to postpone the oral proceedings or to hold them by videoconference could not be granted.

Request of reimbursement of the appeal fee

10. With their notice of appeal, the appellant requested the reimbursement of the appeal fee.

In a communication pursuant to Article 17(1) RPBA 2020, the parties were informed that this request was not substantiated.

The appellant provided no arguments in reply to the board's communication. No reasons can be seen for an assessment different from the one made in the board's communication.

Main request (claim as granted)
Article 100(c) EPC

11. The appellant asserted that the method of claim 1 comprised a step (a) of *"providing a solid support comprising a polynucleotide template"*, whilst the parent application disclosed a step of "providing a polynucleotide template having a first free 3' hydroxyl group which is positioned to initiate sequencing of a first region for sequencing determination on a first template strand". The solid support of granted claim 1 was defined to "comprise" a polynucleotide template, while the parent application specified that the solid support was either "linked" to the polynucleotide template or to the linker polynucleotide (see claim 3).

11.1 Methods for pairwise sequencing of first and second regions are disclosed in Figures 3 to 6 and the corresponding paragraphs of the description, beginning at page 13 of the parent application.

11.2 The method of claim 1 comprising a step (a) of "providing a solid support comprising a polynucleotide

template" finds an adequate basis in the parent application:

11.3 First, even if claim 3 of the parent application specifies that the polynucleotide template or the linker polynucleotide has to be "linked" to the solid support, the method for sequencing a double-stranded template, illustrated in Figure 3 and described on page 16 lines 14 and seq., mentions explicitly that the formation of the starting polynucleotide template and sequencing of the first region of the template proceeds as described for the embodiment shown in Figure 1 (see page 13, lines 11 to 19). This section states that linkage to the solid support could be provided via the primer or the template strand itself. If linkage to the solid support is provided via the template strand then this may occur at the 5' or the 3' end of the template strand, or even via an internal portion of the template provided that this does not interfere with subsequent sequencing reactions (see page 13, line 29 to page 14, line 3). Finally, "[T]he following preferred features apply mutatis mutandis to all embodiments of the invention: ... Templates might be attached to a solid support via any suitable linkage method known in the art. Preferably linkage will be via covalent attachment." (see page 26, lines 30 to 31 and page 28, lines 19 to 20). This disclosure forms an adequate basis for a method step a) providing a solid support comprising a polynucleotide template.

11.4 Secondly, the method of claim 1 step (a) requires neither an explicit nor an implicit presence of a first free 3' hydroxyl group, this step does not exclude embodiments where a solid support comprising a polynucleotide template lacks a first free 3' hydroxyl group (e.g. when the polynucleotide template is bound

to the solid support either by its own 3' end or by its covalently linked flanking sequence 3' end). If the polynucleotide template is bound to the solid support via its 3' end or via the 3' end of a polynucleotide linker sequence linked to the 3' end of the polynucleotide template, then the extension of a polynucleotide chain complementary to the polynucleotide template region will occur only if a primer capable of annealing to the template polynucleotide provides the missing 3'-OH end.

11.4.1 Any covalent or non-covalent, direct or indirect attachments fall under this linkage definition as the template might be bound by any suitable linkage method known in the art, preferably via covalent attachment (see parent application, page 28 lines 19 to 20). Claim 1 does not require the template strand to be directly linked to the solid support. Any oligonucleotide primer hybridizing to the template strand could also provide an initiation point for sequencing, should no free 3'-OH group be provided by the template (see parent application, page 13 lines 26 to 28). Even if claim 1 covers methods using a polynucleotide template (1) consisting of a template strand (3) ligated to a self-complementary hairpin linker without a free 3'-OH group (4), wherein said linker is linked to the solid support, it cannot be said that for these embodiments only the primers are allowed to be linked to the solid support. The "Linkage to the solid support could be provided via the primer or via the template strand itself." (see parent application on page 13 lines 29 to 30). Moreover, the term "via" in this sentence covers both direct and indirect linkages to the solid support. Thus, the appellant's embodiment using a polynucleotide template, consisting of a template strand and a hairpin linker without free 3' -OH group, correspond to an

embodiment in which a template strand itself is bound to the solid support by its covalently bound hairpin linker and via its template strand. There is no disclosure in the parent application that a primer having a free 3'-OH group has to be bound to the solid support if a method uses a template strand bound to the solid support via either its own 3' end or a hairpin linker lacking a free 3'-OH group (see parent application, page 13, lines 26 to 28). Thus, step a) of claim 1 has a basis in the parent application.

11.5 Thirdly, the appellant argued that there was no direct and unambiguous disclosure in the parent application of step (c) of claim 1 which required "extending the strand complementary to the first template strand by ***sequential addition of further nucleotides, which do not contain any labels required for sequencing,*** to the last nucleotide added in step (b), ***thereby defining a second region*** *complementary to the first template strand*".

11.6 The parent application describes a method for sequencing two regions of a polynucleotide template, referred to as the first and second regions for sequence determination, which are on complementary strands of a double-stranded or self-complementary polynucleotide template (see page 7, lines 4 to 11; Figures 3 to 6). The formation of the starting polynucleotide template and sequencing of a first region of the template (7) proceeds as described for the embodiment shown in Figure 1. Once the first sequencing reaction is deemed complete, sequencing of a second region of the polynucleotide template can be carried out. For a second sequencing reaction it is necessary to generate a second free 3' hydroxyl group which serves as an initiation point to sequence a

second region of the template (see paragraph bridging pages 10 and 11). In the alternative, after the first sequencing reaction is deemed to be complete, further nucleotides are added to the 3' hydroxyl group of the last nucleotide added in the first sequencing reaction in order to fully extend the complementary strand (10) to the full length of the first template strand (3) (see Figure 3). This extension reaction proceeds without sequencing, i.e. without determination of the nature of the added nucleotides, and results in the production of a blunt-ended fully double-stranded template ligated at one end to a hairpin linker (4), which itself is linked to a solid support (5) (see Figure 3B; page 16, lines 11 to 30 and lines 20 to 27).

11.6.1 From the recited sections, the skilled person derives that after sequencing a first region, an extension reaction without sequencing must occur, i.e. without determination of the nature of the added nucleotides, resulting in the production of a blunt-ended fully double-stranded template ligated at one end to a hairpin linker. Although there is no indication that the added nucleotides must be free of any labels required for sequencing, the initial step referred to in Fig 3A proceeds as described on page 14 for Figure 1, which includes the subsequent extension step. This nucleotide addition step occurs without sequencing, using nucleotides preferably "...'unmodified', meaning that they do not contain any labels required for sequencing, e.g. fluorescent labels, or any blocking groups" (see page 14, lines 11 to 24). This view is supported in generic terms on page 16, lines 25 to 30).

11.6.2 The method for pairwise sequencing of claim 1 defines that the "second region" whose sequence has to be determined in step (e) to follow the first sequenced

region and to be *complementary to the first template strand*. Both passages on page 10, line 29 to page 11, line 26 and on page 17, lines 19 to 22, define that "a" - undefined - first region and "a" - undefined - second region of the first and the second template strand are sequenced.

For a double-stranded polynucleotide template or a single polynucleotide molecule which is partially complementary, the parent application specifies that "the second free 3' hydroxyl group must be provided on a polynucleotide strand which is base-paired to the second template strand to be sequenced in the region immediately upstream of the second free 3' hydroxyl group. The second template strand overhangs the second free 3' hydroxyl group to define a second region of the template to be sequenced" (see page 11, lines 6 to 11). From this disclosure, the skilled person derives that the first and second regions to be sequenced cannot be on the same template strand for a double stranded polynucleotide template. The second region to be sequenced in step (c) must therefore be located 5' from the 3' end of the second template strand. The method of claim 1 step d) requires then that the 3' end of the second template strand is ligated to the 5' end of a self complementary hairpin linker polynucleotide comprising a free 3' hydroxyl group at its 3' end. The free 3' hydroxyl group at the end of the ligated second hairpin linker provides the initiation point for the second sequencing reaction of step e) of the method of claim 1. As a result, the second template strand and the region to be sequenced overhang the free 3' hydroxyl group of the hairpin linker. Hence, the sequencing as defined in step (e) of claim 1 of "a" second region located 5' on the second template strand

finds a basis in the parent application on page 10, line 29 to page 11, line 26.

11.7 Thus, Article 100(c) EPC does not prejudice maintenance of the patent.

Sufficiency of disclosure (Article 100(b) EPC)

12. The appellant asserted that claim 1 lacked technical details as to how the sequencing reaction was carried out. It failed to define in what orientation the template had to be attached to the solid support, that it required a free 3'-OH group for carrying out the sequencing-by-synthesis reaction of step (b) and where and how the first nucleotide could hybridize to in the sequencing reaction.

If several primers were added to the polynucleotide template, immobilized or not, this could have generated partial doubled-stranded templates that prevented the extension reaction required in step (c) due to their hybridization location. If an internal primer was used in the sequencing reaction, the sequencing of the whole strand, depending on the length of the polynucleotide template strand, would be unfeasible thereby preventing the subsequent step of the method as well. It was accordingly neither sufficiently disclosed how the incorporated nucleotides were detected nor whether and how the labels of the nucleotides were removed nor how the number of incorporated nucleotides was determined and how many nucleotides were added. The extension reaction could therefore cover the full strand resulting in a double-stranded nucleotide which could not be sequenced as required in step (e) of claim 1, unless the first polynucleotide template strand was removed. This step was missing in claim 1.

13. An objection under Article 100(b) EPC presupposes that serious doubts substantiated by verifiable facts exist that the invention can be readily put into practice. The burden of proof rests generally on the opponent (see Case Law of the Boards of Appeal, 9th edition, 2019, II.C.9).

13.1 The appellant asserted that when applying sequencing-by-synthesis (SBS) the skilled person was unable to remove the labels from the nucleotides or to determine the number of added nucleotides and thus to reproduce the method of claim 1. It was further unclear how the detection of incorporated nucleotides could be performed at the rate of processivity of a polymerase.

14. However, a detailed disclosure is not necessary if the skilled person, who has common general knowledge at the immediate disposal, is capable of putting the invention into practice without undue burden. The cited background art supports the view that the skilled person is familiar with the minimal and basic technical requirements associated with the sequencing-by-synthesis (SBS) techniques (see paragraphs [0003] and [0005]). Thus, absent verifiable facts supporting appellant's objections of sufficiency of disclosure, these arguments are merely speculative and cannot convince the board.

14.1 The skilled person was aware how and in which orientation the polynucleotide template can be immobilized on the solid support, and that the sequencing of very large nucleotide sequences beyond a certain limit was unfeasible under some circumstances, as the sequencing read length was dependent on the sequencing method, the polynucleotide sequence itself,

its quality, the separation and detection methods used. The skilled person knew which sequencing read lengths apply to prior art sequencing techniques. Thus, depending on the orientation of the immobilized polynucleotide template on the solid support and on whether it provides a free 3'-OH group as initiation point on the immobilized polynucleotide template, the skilled person knew that said free 3'-OH group must be provided, be it by the polynucleotide template strand or a primer, as described in the patent.

14.2 The method of claim 1 "comprises" at least the steps of claim 1. This wording does not exclude additional steps, such as adding a primer, as mentioned for example in paragraph [0033] of the patent, when no free 3-OH group is available as initiation point for the sequencing of the first region of step (b). Hence neither the sequencing-by-synthesis (SBS) method, known as a standard sequencing technique, nor the missing 3'-OH group in claim 1, prevented the skilled person from carrying out the method of claim 1 without undue burden. The appellant has failed to demonstrate that the skilled person was incapable of determining the number of added nucleotides introduced during the extension step.

There was also no evidence that a full double-stranded nucleotide template ligated to a hairpin linker could not be sequenced by a strand displacing polymerase enzyme, if necessary in combination with a nicking enzyme (see Figures 3 to 6).

Furthermore, a method wherein the first region to be sequenced is the entire first template strand, thereby rendering the extension step c) of claim 1 impossible, would not fall under the scope of claim 1 because

there would be no second region of the template to be sequenced left on the complementary strand of the first template polynucleotide sequence.

14.3 The skilled person would have interpreted claim 1 with a mind willing to understand, excluding illogical, unnecessary or detrimental steps that do not serve the purpose of the claimed method. If the skilled person decided to nonetheless add several primers to the polynucleotide to be sequenced or to add only one primer for sequencing an extremely long template strand, generating thereby partially or fully doubled-stranded templates, they, for this very reason, could not carry out the extension step c) of the method of claim 1 and consequently could not sequence the second region as defined in step e). In consequence, such methods are not embodiments of the method of claim 1.

14.4 Consequently, Article 100(b) EPC does also not prejudice maintenance of the patent.

Inventive step (Article 100(a) and Article 56 EPC)

Closest prior art

15. It was common ground between the parties that document D1 represents the closest prior art for the method of claim 1. It discloses the sequencing of two single-stranded nucleic acid templates derived from a double stranded template where each of the single-stranded templates is immobilized on a solid support. Two primers (one blocked and one unblocked) are used to sequence both ends of a double stranded nucleic acid. Even if a second region complementary to the first template strand may be defined by extending the first template strand, there is no disclosure in document D1

of the ligation of a hairpin linker polynucleotide comprising a free 3' hydroxyl group to the free 3' end of the extended strand from which the sequencing reaction of the second region is carried out. Claim 28 discloses at best a method wherein the second sequencing primer is deblocked and at least one base is incorporated into said second strand by extending said second primer with a polymerase.

15.1 The method of claim 1 differs from that of document D1 in that it relates to a method for pairwise sequencing of first and second regions on complementary strands of a double-stranded polynucleotide template immobilized on the solid support, in that a hairpin linker instead of a primer is used for sequencing the second template strand, and no emulsion PCR is used.

15.2 The technical effect underlying these differences is that it reduces the number of primers needed (no multiplicity of primers needed for sequencing the second region) which makes the method more robust.

15.3 Starting from document D1, the technical problem may be formulated as the provision of an improved sequencing method for pairwise sequencing of double stranded polynucleotide templates.

15.3.1 The method according to claim 1 solves this problem.

Obviousness

15.4 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.

15.5 The appellant asserted that document D1 provided an incentive to look for an improved method as it explicitly stated that the sequencing based on chemical cleavage was difficult to automate. Other sequencing methods were labor intensive due to the need to perform a hybridization step for every sequencing effort (see page 2, lines 18 to 21).

Document D3 taught the sequencing of polynucleotide templates forming part of an array which were immobilized by hairpin linkers to a solid support. The immobilization of the templates on a solid support was achieved by covalent or non-covalent binding of the templates at their 5' or 3' end, or via a linker, which could be a hairpin loop structure (see page 6, lines 11 to 29). The determination of the sequence was carried out by the incorporation of a labelled base at pre-determined positions followed by non-labelled bases (page 6, line 29; page 9, lines 20 to 29).

Alternatively, document D19 disclosed a method of amplifying a polynucleotide hairpin using a single primer. The method could be used to amplify double-stranded polynucleotides and to detect hairpin, double-stranded and single-stranded polynucleotides (see abstract, page 1, lines 23 to 25). This method was useful for the amplification and sequencing of unknown regions of DNA flanking regions of known sequence (see page 3, lines 1 to 3, Figure 5, page 3, lines 23 to 28). The ability to bind a primer to the hairpin adapter allowed the determination of the sequence adjacent to the hairpin adapter following hairpin amplification (see page 19, lines 11 to 15). The use of single-stranded DNA or RNA was disclosed on page 20, lines 9 to 11).

16. When considering whether a solution to an objective technical problem was obvious or not, the question to be answered is not whether the skilled person could have carried out the invention, but whether they would have done so in the expectation of solving the underlying technical problem or in the expectation of some improvement or advantage (decision T 2/83, OJ 1984, 265)

16.1 Document D1 discloses that there was a need to improve sequencing methods (see section entitled "Background", page 2, lines 18 to 21). This problem was solved in document D1 by a method using single-stranded templates and blocked and unblocked primers. The solution claimed in document D1 is not described as leaving any identifiable technical problems unresolved. There is no indication in document D1 and no pointer how to further improve the sequencing method of claim 1, for instance by adapting and combining it with some of the elements used in the method of document D3, with the additional need to apply it to double-stranded templates.

16.2 Even if the skilled person turned to document D3, the claimed solution was not obvious. Document D3 teaches the use of hairpin linkers only for the immobilization of the template strands but not their addition to the 3'-OH group of an extended strand and the subsequent sequencing of a second region of the extended strand complementary to the first template.

16.3 Document D19 discloses the binding of a hairpin adapter to a double stranded DNA with unknown sequence to enable the amplification of a template strand with only one primer when the strand following the loop of the hairpin is extended (see p.3, lines 26 to 28; Fig.5).

This template is not bound to a solid support and it is only the binding of a second primer to the hairpin adapter, and not of a hairpin linker as claimed, which allows the determination of the sequence adjacent to the hairpin adapter following hairpin amplification.

Thus, even if the skilled person turned to document D19, it would not have arrived at the claimed solution in an obvious way.

17. To conclude, also the finding in the decision under appeal with respect to Article 56 EPC is correct.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

L. Malécot-Grob B. Stolz

Decision electronically authenticated