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Datasheet for the decision of 5 December 2019

Case Number: T 1524/15 - 3.3.08

Application Number: 07705178.7

Publication Number: 1987159

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, 123(2)
RPBA Art. 12(3)

Keyword:
Main request and auxiliary requests 1 to 3 - added subject-matter - (yes)
Auxiliary requests 4 and 5 - inventive step - (no)
Auxiliary request 7 - requirements of the EPC met - (yes)

Decisions cited:
T 0823/96, T 0860/00, T 1072/07, T 0425/09

Catchword:

Europäisches Patentamt
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Office européen des brevets

Beschwerdekammern

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Case Number: T 1524/15 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 5 December 2019

Appellant I: (Patent Proprietor)	Illumina Cambridge Limited 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)
Appellant II: (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 8 June 2015 concerning maintenance of the European Patent No. 1987159 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. Montrone
	R. Winkelhofer

Summary of Facts and Submissions

I. Appeals were lodged by the patent proprietor (hereinafter "appellant I") and the opponent (hereinafter the "appellant II") against the decision of an opposition division to maintain the European patent No. 1 987 159 in amended form. The patent was filed under the PCT and published as international patent application WO 2007/091077 (hereinafter the "patent application") having the title "Method for sequencing a polynucleotide template".

II. The opposition division held in the decision under appeal that the subject-matter of claims 1 of the main request (claims as granted) and of auxiliary requests 1 to 3 contravened Article 123(2) EPC; that the subject-matter of claims 1 of auxiliary requests 4 and 5 lacked clarity; and that the subject-matter of claim 1 of auxiliary request 6 lacked novelty over the disclosure of document D5. Auxiliary request 7 was found to meet the requirements of the EPC.

III. During the appeal proceedings, appellant I submitted a main request (claims as granted) and auxiliary requests 1 to 8.

IV. Appellant II contested the patentability of these requests as they contravened Article 123(2) EPC, or were insufficiently disclosed, lacked novelty or an inventive step.

V. 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, appellant II

withdrew the request for oral proceedings, and announced that it would not be attending them, without submitting substantive arguments in response to any of the issues raised in the board's communication.

VI. Oral proceedings before the board were held on 5 December 2019, in the absence of appellant II. At the oral proceedings appellant I withdrew auxiliary request 6.

VII. Claims 1 of the main request (claims as granted) and of auxiliary requests 1 to 3 read as follows:

"1. A method for pairwise sequencing of first and second regions of double stranded polynucleotides immobilised on a solid support, wherein said first and second regions are in the same target double stranded polynucleotide and wherein the target double stranded polynucleotides remain immobilised to the solid support, the method comprising:

(a) providing a solid support having immobilised thereon the double stranded polynucleotides each formed from complementary first and second template strands;

(b) treating the plurality of double stranded polynucleotides to denature said double stranded template polynucleotides to allow hybridisation of a sequencing primer to the first or second template strands;

(c) hybridising the first sequencing primer to the first or second template strands generated in part (b);

(d) carrying out a first sequencing reaction to monitor the incorporation of nucleotides or oligonucleotides

onto the first sequencing primer using primer extension to generate a first extended sequencing primer and determine the sequence of a first region of the template polynucleotide;

(e) removing the extended sequencing primer from step (d);

(f) hybridising the second sequencing primer to the template strand of step (c) or a complement thereof; and

(g) carrying out a second sequencing run to monitor the incorporation of nucleotides or oligonucleotides onto a second sequencing primer using primer extension to generate a second extended sequencing primer and determine the sequence of a second region of the template polynucleotide, wherein determining the sequence of the first and second regions of the template polynucleotide achieves pairwise sequencing of said first and second regions of said target double stranded polynucleotide."

VIII. Claims 1 of auxiliary requests 4 and 5 differ from claim 1 of the main request in that the features "*both strands of*" and "*linked to the solid support at their 5' ends*" have been added to the preamble and step (a), respectively.

IX. Claim 1 of auxiliary request 7 differs from claim 1 of the main request in that the preamble and step (a) has been amended as in auxiliary requests 4 and 5 (see above). In addition, step (f) has been amended in that the feature "*template strand of step (c)*" was replaced by "*complement of the template strand of step (c)*", and

in that the feature "*or a complement thereof*" was deleted.

Claim 9 of auxiliary request 7 reads:

"9. The method according to claim 8, wherein the known primer region contains a site recognised by a restriction enzyme, wherein treating of step (b) is performed using said restriction enzyme to denature the target double stranded polynucleotide, and heat or chemical denaturation is used to remove non-covalently attached single stranded polynucleotide regions from the surfaces."

Claims 2 to 8 and 10 to 13 define specific embodiments of the method of claim 1.

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

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

D2: WO 97/18328 (published 22 May 1997);

D3: G.-H. Zhou *et al.*, Nucleic Acids Research, 2005, Vol. 33(15), e133, 1-11;

D5: EP-A-1 591 541 (published 2 November 2005).

XI. Appellant I's submissions, insofar as relevant to the present decision, may be summarised as follows:

Main request and auxiliary requests 1 to 3

Added subject-matter - claim 1

Omission of the feature "linked to the solid support at their 5' ends" from claim 1

Step (a) of claim 1 as granted lacked the feature "*linked to the solid support at their 5' ends*", which was present in claim 1 of the application as filed. Since step (a) did no longer define the way in which double-stranded polynucleotides were immobilised to a solid support, they could be immobilised in any possible manner, i.e. not at their 5'-ends only. Support for this amendment was found on page 12, lines 15 to 26 of the patent application which referred in general to the immobilisation of nucleic acids on solid supports; the paragraph bridging pages 27 and 28 of the patent application, which disclosed that a double-stranded DNA was immobilised at only one end of the duplex; and on page 57, lines 15 to 18, of the patent application which referred to double-stranded templates that were immobilised on a support in general.

Addition of the feature "oligonucleotides" in steps (d) and (g) of claim 1

The feature was implicitly disclosed on page 47, lines 15 to 23 of the patent application, which mentioned sequencing by ligation-based methods that necessarily involved the incorporation of oligonucleotides during the sequencing reaction.

Auxiliary requests 4 and 5

Claim construction - claim 1

The term "*pairwise sequencing*" referred to in claim 1 implied that the nucleotide sequence was determined sequentially in two distinct and separate regions on

either one or both strands of a double-stranded polynucleotide target.

The feature "*wherein both strands of the target double stranded polynucleotides remain immobilised to the solid support*" in the preamble of claim 1, implied that both strands remained immobilised throughout the sequencing process, but not that both strands remained in a double-stranded form immobilised during that process. This was not in line with steps (b), (c), (e) and (f) of claim 1 which required a denaturation of the double-strands, or the removal of the complementary extended strand prior to the hybridisation of a first and a second sequencing primer to one of the two target strands. Such a hybridisation necessarily implied that the strands were single-stranded or at least partially single-stranded.

The term "*complement of the template strand*" cited in step (f) of claim 1 was not to be interpreted literally in the sense that the second sequencing primer needed to hybridise to a sequence that was complementary to the first template strand, but rather that it hybridised to a strand that was the complement of the first strand in a double-stranded polynucleotide.

Inventive step - claim 1

The method of claim 1 was *inter alia* directed to the pairwise sequencing of at least two regions of a double-stranded polynucleotide immobilised on a solid support based on a sequential hybridisation of different primers, primer extension and removal of an extended sequencing primer after the first sequencing reaction, wherein the regions to be sequenced were located on one of the two strands of the duplex only.

Document D1 represented the closest prior art, since it disclosed a method for pairwise sequencing of immobilised target polynucleotides, i.e. it was directed at the same purpose as the method of the invention.

Document D5 did not represent the closest prior art. It was primarily concerned with methods for a bridge amplification of immobilised nucleic acids. As regards sequencing, the document disclosed as targets immobilised single-stranded polynucleotides only, wherein the other strand of the duplex was removed prior to sequencing. Although document D5 mentioned in paragraph [0201] a re-sequencing approach based on a sequential hybridisation of different primers followed by primer extensions, this approach could be equated with a pairwise sequencing as referred to in claim 1 by hindsight of the invention only, since re-sequencing was not applied in general to immobilised single-stranded target nucleic acids, but solely under special circumstances dealing with long molecules. Thus, the sequencing method of document D5 concerned a different purpose than the claimed method.

If document D5 represented the closest prior art, the claimed method differed therefrom in that the second strand remained on the solid support during the sequencing reactions because it was immobilised, while in document D5, one of the two complementary strands was removed prior to sequencing. The presence of both complementary strands during sequencing offered the skilled person more options regarding the target strand to be sequenced. The technical problem to be solved was thus the provision of a more versatile sequencing method.

The solution provided by the method of claim 1 wherein both strands remained immobilised on a solid support instead of one, was not obvious to the skilled person since none of the cited prior art documents provided hints at keeping both strands immobilised to have more sequencing options at hand.

Auxiliary request 7

Sufficiency of disclosure - claim 9

The subject-matter of claim 9 was dependent on claim 8, which was dependent on claim 1. Thus, claim 9 *inter alia* required that a primer region located between a first and second region to be sequenced on the target polynucleotide contained a site recognised by a restriction enzyme. The action of the enzyme followed by either heat or chemical denaturation of the double-stranded target polynucleotides generated truncated single-strands, wherein two single-stranded partial sequences remained immobilised to the solid support, while two partial sequences being complementary to the immobilised sequences were removed.

Although as a result thereof, the remaining immobilised single-strands were no longer double-stranded and, due to the loss of their complementary sequence counter-parts, literally not complementary to each other, this was immaterial for sufficiency of disclosure. This was so because the method of claim 1, for the reasons set out above, neither required that both strands of the target polynucleotide remained immobilised in double-stranded form throughout the sequencing process, nor was there a need for a hybridisation of the second sequencing primer in step (f) to a literal

complementary sequence part of the first template strand, but only to a second strand that was the complement of the first in the double-stranded polynucleotide template. These requirements were complied with by the subject-matter of claim 9.

Novelty - claim 1

The subject-matter of claim 1 was novel over the disclosure in document D5, since it described a sequencing method of immobilised single-stranded polynucleotides only, wherein the second complementary strand was removed prior to sequencing. That both of the double-stranded polynucleotides remained immobilised throughout the sequencing process was not directly and unambiguously disclosed in document D5. Furthermore, the document was silent on the sequencing of two regions on both strands of double-stranded polynucleotides, in other words a pairwise sequencing. It was doubtful that paragraphs [0151] to [0159] of document D5 disclosed a sequencing method since the passages rather related to preparation steps of hybridisation assays required for detection purposes.

Inventive step - claim 1

Document D1 represented the closest prior art, since it was the only document that concerned the pairwise sequencing of two complementary strands of a double-stranded polynucleotide. In other words, it was directed to the same purpose as the claimed method.

Document D5 did not represent the closest prior art, since it was not directed to the sequencing of regions on both complementary strands of an immobilised double-

stranded target polynucleotide, but to the sequencing of single-strands only.

The method of document D1 relied on a simultaneous hybridisation of two or more primers to a target polynucleotide. All of the primers were blocked, except one, which was used to start the sequencing reaction. Subsequently, this starting primer was blocked, and one of the previously blocked primers was unblocked to initiate a second sequencing reaction.

The claimed method differed from the closest prior art method in that the extended first sequencing primer referred to in step (e) had to be removed, and in that the sequencing primer hybridisation occurred sequentially. Contrary thereto, the method in document D1 required that all primers hybridised simultaneously, and that extended primers were not removed prior to starting a second sequencing run.

The sequential hybridisation of primers had the effect that during sequencing only one primer was present for extension, and that therefore no laborious and error prone chemical measures were required to avoid unwanted extensions of other primers. The technical problem solved by the claimed method was the provision of a more efficient method for pairwise sequencing.

The method of claim 1 as a solution to this problem was not obvious for the skilled person starting from document D1, since the document was silent on motivations to change the simultaneous primer hybridisation followed by cycles of blocking/unblocking those primers, and provided no hints to instead hybridise the primers sequentially.

The method of claim 1 was also not obvious when the teaching of document D1 was combined with that of document D5.

The method disclosed in document D5 was directed to a sequencing of immobilised single-stranded target polynucleotides, wherein the complementary second strand was removed prior to sequencing (see e.g. Figure 16(e)). Thus, the second strand was not available for sequencing. This was also not derivable from paragraphs [0158], [0177] and [0201] in document D5. Paragraph [0158] in document D5 related to probe hybridisation steps but not to sequencing, let alone to the sequencing of the second complementary strand. Nor was the use of complementary strands as templates for sequencing disclosed in paragraphs [0151] to [0159] of document D5. Paragraph [0177] in document D5 stated explicitly that the sequencing started "*once single-stranded molecules to be sequenced are provided*". Thus, depending on which strand was removed prior to sequencing, only one of the two PCR amplification primers was used for sequencing. The sequencing method of document D5 neither required nor intended/suggested the sequencing of regions located on both strands of a template like the claimed method. Paragraph [0201] in document D5 concerned specific embodiments wherein a sequencing run could be repeated. It mentioned the sequencing reactions discussed above, but was silent on sequencing the complementary strand. Therefore, document D5 did not hint at the use of the complementary strand for sequencing. Document D5 rather taught away from sequencing both strands of a template, since it explicitly proposed the removal of the second strand prior to sequencing for an efficient sequencing of regions located on the same strand.

XII. Appellant II's written submissions, insofar as relevant to the present decision, may be summarised as follows:

Main request and auxiliary requests 1 to 3

Added subject-matter - claim 1

Omission of the feature "linked to the solid support at their 5' ends" from claim 1

The patent application did not provide a basis for the omission of the feature *"linked to the solid support at their 5' ends"* in claim 1, for the reasons set out in the decision under appeal.

Addition of the feature "oligonucleotides" in steps (d) and (g) of claim 1

Claim 1 of the patent application disclosed that nucleotides were used for carrying out the sequencing reaction, since it referred to "*using cycles of primer extension with a polymerase and labeled nucleotides*" which was incompatible with the use of oligonucleotides for sequencing. Also page 47, lines 17 to 19 of the patent application provided no basis for the use of oligonucleotides, since the disclosed sequencing method was restricted to one "*which relies on successive incorporation of nucleotides into the polynucleotide chain*", which was incompatible to an incorporation of oligonucleotides, and hence, contradictory to ligation-based methods likewise disclosed on page 49. A similar situation was encountered in case T 0425/09 in which the board held that an amended feature for which the basis in the description was contradictory was not directly and unambiguously disclosed in the application as filed.

Auxiliary requests 4 and 5

Inventive step - claim 1

Documents D1 and D5 represented both the closest prior art.

The sequencing method disclosed in document D1 relied on the simultaneous use of blocked and unblocked sequencing primers. In view of the distinguishing features and the effect achieved when compared to the claimed method, the technical problem to be solved was regarded as the provision of a more efficient pairwise sequencing method.

The claimed method was obvious for the skilled person combining the teaching of documents D1 and D5. The skilled person looking at document D1 and the technical problem defined above immediately recognised that the method disclosed therein could be improved due to the obvious drawbacks caused by the need for blocked and unblocked sequencing primers (see page 9, lines 3 to 9 of document D1). Thus, the skilled person was motivated to look for sequencing-by-synthesis reactions without such a need.

Document D5 disclosed an example of such a reaction, because it described the addition of a second sequencing primer subsequent to the first sequencing run followed by strand denaturation and a primer hybridisation. Document D5 further indicated second rounds of sequencing (see paragraphs [0158] and [0201]), and that the use of PCR amplification primers, which by definition bound to complementary strands, was preferred for sequencing (see paragraph [0177]). The

use of a second primer for sequencing regions on the complementary strand, potentially missing in document D5, was already known from document D1.

The expectation of success was high that a substitution of a blocking/de-blocking cycle of primers by a denaturation/hybridisation cycle allowed the sequencing of both strands of a target molecule, because the method in document D1 relied on cyclic processes too. The claimed method was thus obvious for the skilled person.

In a further line of argument, it was submitted that the skilled person was very well aware that two sequencing runs on different parts of one strand led to the same amount of sequence information compared to a sequencing of two complementary strands. Thus both approaches were well-known alternatives for obtaining the same result. However, the selection of one of two-well known alternatives was obvious to the skilled person (see e.g. decision T 1072/07).

In a third line of argument, it was submitted that paragraph [0177] in document D5 reported the use of PCR primers for sequencing. Although this paragraph indicated that single-stranded templates were provided, the sequencing of the complementary strand was not excluded, because the method did not require a complete removal of the complementary strand, as evidenced by the claimed method itself. Further as set out above, document D5 suggested the use of PCR amplification primers for sequencing too. Thus, the skilled person would have inferred from paragraph [0177] in document D5 that in cases where two sequencing runs were performed in a single colony (see paragraph [0201]), and denatured double-stranded DNA molecules with two

immobilised strands were the target (see paragraph [0155]), a PCR-based sequencing was most straightforward, wherein the first and the second PCR primers sequenced the first and the second (complementary) strands respectively. Moreover, since PCR primers annealed necessarily to complementary strands, both strands were sequenced. Thus, the claimed method was obvious.

Even if argued that document D5 did not provide an incentive for the skilled person to use a second PCR primer for sequencing, it taught at least that a further sequencing primer was used for sequencing a second region on the same strand (see paragraph [0187], lines 29 and 30). In this situation it was evident to the skilled person that a hybridisation of the second primer to a target was possible only if the sequence information of at least a part of the target strand was available. In such a situation, the skilled person would have looked for solutions that did not require any prior knowledge of the sequence. Documents, such as D1 and D5, concerned with paired end sequencing provided a solution to this problem, since they disclosed that sequencing started from both ends of a nucleic acid previously amplified by PCR primers of a known sequence. The later adaptation of a PCR amplification method to be used for sequencing was not technically challenging to the skilled person, and had a high expectation of success.

Document D5 did also not teach away from using complementary strands as templates for a second sequencing, since their removal was an option only (see paragraph [0177]). On the contrary, the document suggested *inter alia* the use of heat for denaturing a double-strand, which left both strands immobilised to

the solid support (see paragraph [0155]). Likewise the sequencing of single-stranded templates did not teach away from sequencing two regions on complementary strands, since for sequencing a double-stranded template, the strands had to be single-stranded anyway.

Auxiliary request 7

Added subject-matter - claim 1

The patent application did not provide a basis for the omission of the feature "*linked to the solid support at their 5' ends*" and the addition of the feature "*oligonucleotides*" into claim 1 for the reasons set out above.

Sufficiency of disclosure - claim 9

The strands according to claim 9, which depended on claim 8 and hence likewise on claim 1, remained neither double-stranded nor complementary immobilised after treatment with a restriction enzyme and chemical/heat denaturation. Thus, the requirements of claims 1 and 9 were incompatible with each other, and the subject-matter of claim 9 insufficiently disclosed.

Novelty - claim 1

The method of claim 1 lacked novelty over the disclosure in documents D1 to D3 and D5.

As regards documents D1 to D3, appellant II submitted that they were detrimental to the novelty of claim 1 for arguments "*described in length in our submissions during opposition proceedings and the Board is referred*

to previously filed document for details of the arguments".

As regards document D5, it was submitted that the features of step (a) in claim 1 were disclosed in Figures 1 and 5; the features of step (b) were disclosed in paragraph [0155]; the features of steps (c) and (d) were disclosed in paragraph [0168]; the features of steps (e) and (f) were disclosed in paragraphs [0187], [0201] and [0177]; and the features of step (g) were in essence identical to those recited in step (d), except for using another sequencing primer for the second sequencing run which was disclosed in paragraph [0187]. Furthermore, since paragraph [0177] in document D5 indicated that the sequencing primers preferably bound to the same sequences as the PCR primers, and it was common general knowledge that PCR primers bound to complementary strands, the passage provided a direct and unambiguous disclosure that the second PCR primer hybridised to the complementary strand of the first strand. The disclosure in paragraph [0189] of document D5 did not limit the sequencing to one strand only, since the passage started with "*The present invention therefore also includes within its scope...*", wherein the term "*also*" implied that other embodiments were not excluded, for example, a primer hybridisation to both strands. An exclusion of a sequencing of both complementary strands was likewise not derivable from paragraphs [0175] and [0176] in document D5, since a digestion of double-stranded templates by a restriction enzyme to provide a single-strand to be sequenced, did not require the removal of the complete complementary strand, as this was merely one of several options. That, for example, both strands remained immobilised to the solid support was suggested by the use of a heat denaturation step in paragraph

[0155] of document D5. A sequencing of complementary strands was implied by the disclosure of paragraphs [0151] to [0159] of document D5, which related to a sequencing method. Thus, these paragraphs in document D5 disclosed all of the features mentioned in claim 1.

Inventive step - claim 1

The subject-matter of claim 1 lacked an inventive step for the reasons set out above for auxiliary requests 4 and 5.

XIII. Appellant I requests that the decision under appeal be set aside and that the patent be maintained either on the basis of the main request (claims as granted, i.e. the opposition be rejected), or in the alternative, on the basis of one of auxiliary requests 1 to 5 or 7 to 8.

XIV. Appellant II requests that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Main request (claims as granted)

Added subject-matter - claim 1

1. Several issues were raised by appellant II under Article 100(c) EPC against the method of claim 1.

Omission of the feature "linked to the solid support at their 5' ends" from claim 1

2. The first issue concerns the question whether or not the absence of the feature *"linked to the solid support at their 5' ends"* in claim 1 as granted, which was present in claim 1 of the patent application, extends the claimed subject-matter beyond the content of the application as filed.

3. Step (a) of claim 1 reads as follows: "*providing a solid support having immobilised thereon the double stranded polynucleotides each formed from complementary first and second template strands*". Thus, step (a) encompasses double-stranded polynucleotides immobilised in any manner to a solid support, because the kind of immobilisation is not defined. Accordingly, nucleic acids to be sequenced having both strands immobilised at any position within the sequence, for example, at their 5'- or 3'-ends, or, molecules immobilised at only one strand fall within the scope of claim 1.

3.1 Appellant I indicated as a first basis for the immobilised double-stranded polynucleotides in step (a) of claim 1, page 12, lines 15 to 26, of the patent application which read as follows: "*When referring to immobilisation or attachment of molecules (e.g. nucleic acids) to a solid support, the terms "immobilised" and "attached" are used interchangeably herein and both terms are intended to encompass direct or indirect, covalent or non-covalent attachment, unless indicated otherwise, either explicitly or by context. In certain embodiments of the invention covalent attachment may be preferred, but generally all that is required is that the molecules (e.g. nucleic acids) remain immobilised or attached to the support under the conditions in*

which it is intended to use the support, for example in applications requiring nucleic acid amplification and/ or sequencing" (emphasis added).

3.2 This passage of the patent application does not refer to the method of claim 1 and has thus to be read in conjunction with the preceding statement on page 11, lines 23 to 32 of the patent application. The latter specifies the "*starting point of the method of the invention*", and states explicitly that the "*duplexes are each formed from complementary first and second template strands which are linked to the solid support at or near to their 5' ends*". Thus, when both passages indicated above are read in conjunction, the patent application provides support for double-stranded polynucleotides that are immobilised to a solid support at both of their 5'-ends only.

3.3 The second basis indicated by appellant I was the paragraph bridging pages 27 and 28 of the patent application which states: "*Polynucleotide molecules can be prepared to contain sequences for two sequencing primers as described above. If such molecules are immobilised such that one of the two immobilised ends can be cleaved from the surface, upon such cleavage the resulting double stranded DNA, which is now immobilised at only one end of the duplex, can be made single stranded using heat or chemical denaturing conditions to give a single stranded molecule containing two primer hybridisation sites. [...]*" (emphasis added).

3.4 This passage discloses that the polynucleotides are immobilised on a solid support in such a way that one of the two immobilised strands can be cleaved, which implies that prior to cleavage, the ends of both strands are bound to a solid support at their 5'-ends

(see page 28, line 16). Furthermore, this passage of the patent application relates to the linearisation of immobilised double-stranded nucleic acids to generate immobilised single-strands, while the preamble of claim 1 requires the immobilised nucleic acids to remain double-stranded. In other words, the cited passage relates to subject-matter not covered by the claim. Therefore, it cannot serve as basis for amended step (a) of claim 1 either.

3.5 The third passage in the patent application relied on by appellant I on page 57, lines 15 to 18, reads as follows: "*The method may be applied to sequencing of double-stranded templates immobilised on a support by any other means amenable to repeated cycles of hybridisation and sequencing*".

3.6 Although this general statement refers to an immobilisation of templates in more general terms, it does not directly and unambiguously disclose double-stranded templates immobilised other than at their 5'-ends.

4. Consequently, none of the passages cited by appellant I provide a basis for the subject-matter defined in step (a) of claim 1.

Addition of the feature "oligonucleotides" in steps (d) and (g) of claim 1

5. It is undisputed that the feature "*oligonucleotides*" is not explicitly mentioned in the patent application. It has thus to be assessed whether or not the patent application provides an implicit disclosure of this feature. According to the established case law, an implicit disclosure is what any person skilled in the

art would consider as necessarily implied by the patent application as a whole, or in other words a clear and unambiguous consequence of what is explicitly mentioned (see Case Law of the Boards of Appeal of the EPO, 9th edition, 2019 (hereinafter "Case Law"), II.E.1.3.1, in particular decisions T 860/00 of 28 September 2004 and T 823/96 of 28 January 1997 cited therein).

5.1 The relevant passage on page 47, lines 15 to 23 of the patent application states: "*The methods of the invention are not limited to use of the sequencing method outlined above, but can be used in conjunction with essentially any sequencing methodology which relies on successive incorporation of nucleotides into a polynucleotide chain. Suitable techniques include, for example, Pyrosequencing™, FISSEQ (fluorescent in situ sequencing), MPSS (massively parallel signature sequencing) and sequencing by ligation-based methods, for example as described in US6306597*" (emphasis added).

5.2 In this context it is further uncontested that any nucleic acid sequencing based on "*sequencing by ligation*" methods necessarily involves the incorporation of oligonucleotides, or in other words, that the incorporation of oligonucleotides is an unambiguous consequence of any sequencing-by-ligation-based method.

5.3 Appellant II submitted that there was a contradiction between ligation-based sequencing methods using oligonucleotides, and a "*sequencing methodology which relies on successive incorporation of nucleotides into a polynucleotide chain*" (emphasis added), i.e. a sequencing-by-synthesis methodology, since only nucleotides, but not oligonucleotides could be

incorporated by the method of the invention. According to appellant II, a similar situation was assessed in decision T 0425/09 of 5 March 2013. There it was held that an amended feature in a claim contravened Article 123(2) EPC if it was based on a contradictory disclosure in the description and it was not evident for the skilled person how that contradiction was to be resolved (see points 2.1.3 to 2.3.4 of the Reasons).

5.4 As set out above, the incorporation of oligonucleotides is an unambiguous consequence of sequencing-by-ligation-based methods as referred to on page 47, line 22 of the patent application. Accordingly, there is no contradiction between the statement "*sequencing methodology which relies on successive incorporation of nucleotides into a polynucleotide chain*" (emphasis added) in the passage above, and the mention of "*oligonucleotides*" in steps (d) and (g) of claim 1, because the incorporation of oligonucleotides instead of nucleotides in "*sequencing by ligation-based methods*", and of nucleotides instead of oligonucleotides in sequencing-by-synthesis methods is immediately apparent to the skilled person. Consequently, the situation dealt with in T 0425/09 differs fundamentally from the present one, and hence, cannot be relied on by appellant II in support of its case on added subject-matter.

6. In light of the considerations above, the board concludes that the feature "*oligonucleotides*" in steps (d) and (g) of claim 1 is directly and unambiguously disclosed in the patent application as a whole.

However, since as set out above, step (a) in claim 1 comprises added subject-matter, claim 1 as a whole, and hence the main request, contravenes Article 123(2) EPC.

Auxiliary requests 1 to 3

Added subject-matter - claim 1

7. Steps (a) of claims 1 of auxiliary requests 1 to 3 lack the feature "*linked to the solid support at their 5' ends*" too. Consequently, the objections under Article 123(2) EPC raised above for the method of claim 1 of the main request apply *mutatis mutandis* to these auxiliary requests.

Auxiliary requests 4 and 5

8. Claims 1 of auxiliary requests 4 and 5 are identical to each other and differ from claim 1 of the main request in that the features "*both strands of*" and "*linked to the solid support at their 5' ends*" have been added to the preamble and step (a), respectively.

Claim construction - claim 1

9. For the assessment of sufficiency of disclosure, novelty, and inventive step, the interpretation of the subject-matter of claim 1 is relevant in the present case.

10. The method of claim 1 is directed to a pairwise sequencing of two separate regions on one strand only (**alternative A**: "*template strand*", see step (f)), or on both strands (**alternative B**: "*complement thereof*", see step (f)) of a single immobilised double-stranded polynucleotide target, wherein both strands are linked to a solid support at their 5'-ends.

The sequencing of both regions is performed in a sequential process, starting with a first denaturation of the double strand, followed by annealing of a first sequencing primer, a first primer extension reaction, and the determination of the sequence of the first region.

These process steps are then repeated to determine the sequence of a second region located either on the same strand (**alternative A**), or on the complementary strand (**alternative B**) by using a second sequencing primer, i.e. a primer that is different from the first primer.

10.1 The term "*pairwise sequencing*" referred to in the preamble of claim 1 relates to a method that sequentially determines the nucleic acid sequences of two distinct and separate regions located either on one (**alternative A**), or on both strands (**alternative B**) of an immobilised double-stranded polynucleotide.

10.2 Double-stranded polynucleotides wherein both strands are immobilised at their 5'-ends to a solid support as referred to in step (a) of claim 1 are characterised by so-called "bridge structures" (see e.g. Figure 8-1 of document D5).

10.3 It is uncontested that the feature "*wherein both strands of the target double stranded polynucleotides remain immobilised to the solid support*" (emphasis added) in the preamble of claim 1, implies that both strands remain immobilised on the solid support throughout the sequencing process. Appellant II submitted that this feature further required that both strands remained immobilised in double-stranded form during that process. The board is not convinced by this argument, since such an interpretation runs counter to

the instructions of steps (b), (c), (e) and (f) of claim 1, which require that the double-stranded target molecules are denatured prior to hybridisation with a first sequencing primer, i.e. become single-stranded, and that the extended strand is removed prior to a hybridisation with a second sequencing primer, i.e. becomes again single-stranded.

10.4 Appellant II further submitted that the term "*complement of the template strand*" cited in step (f) of claim 1 of auxiliary request 7 was to be interpreted in the sense that the second sequencing primer needed to hybridise to a sequence that was complementary to the first template strand. This argument is not convincing either, since the claim does not recite that the primer hybridises to the complementary sequence of the first template strand, in other words to complementary sequences in a strict literal sense, but to *"a complement*". This requires that the second primer hybridises to a strand that represents at least in part the *"complement*" of the first strand of the immobilised double-stranded polynucleotide template before the sequencing starts.

Novelty - claim 1

11. The method of claims 1 of present auxiliary requests 4 and 5 is identical and corresponds to claim 1 of auxiliary request 6 dealt with in the decision under appeal. Appellant II and the opposition division both take the view that the claimed subject-matter lacked novelty over the disclosure of document D5.

12. The issue to be assessed in this context is whether or not document D5 discloses a method of "*pairwise sequencing*" wherein "*both strands of the target double*

stranded polynucleotides remain immobilised to the solid support" throughout the sequencing process. Document D5 does not explicitly disclose this feature.

13. It is a generally applied principle that, for concluding lack of novelty, there must be a direct and unambiguous disclosure, either explicit or implicit, in the state of the art of subject-matter falling within the scope of what is claimed (see Case Law, I.C.4).

14. Document D5 discloses a method of nucleic acid amplification generating preferably complementary double-stranded polynucleotides wherein both strands are immobilised at their 5'-ends in distinct areas (i.e. "*colonies*") on a solid support (see paragraphs [0016] and [0022]). Document D5 further discloses various methods of sequencing these polynucleotides (see abstract, section I(iii): paragraphs [0164] to [0201], claim 1), including sequencing-by-ligation and sequencing-by-synthesis (see paragraphs [0167] and [0168]). The latter method, being preferred, is based on different steps, including primer hybridisations, primer extensions, and sequence determinations by detecting labelled nucleotides in the extended sequence (see paragraphs [0168] and [0174]).

Concerning the sequencing of polynucleotides in single-stranded or double-stranded form, the relevant paragraphs [0175] and [0176] in document D5 read as follows: "*If colonies are provided initially in a form comprising double-stranded molecules the colonies can be processed to provide single-stranded molecules for use in sequencing as described above. (It should however be noted that double stranded molecules can be used for sequencing without such processing. For example a double stranded DNA molecule can be provided*

with a promoter sequence and step-by-step sequencing can then be performed using an RNA polymerase and labelled ribonucleotides (cf. Figure 16 d)). Another alternative is for a nick to be introduced in a double stranded DNA molecule so that nick translation can be performed using labelled deoxyribonucleotides and a DNA polymerase with 5' to 3' exonuclease activity.)

[0176] One way of processing double-stranded molecules present in colonies to provide single-stranded colonies as described later with reference to Figure 19. Here double-stranded immobilised molecules present in a colony (which may be in the form of bridge-like structures) are cleaved and this is followed by a denaturing step. (Alternatively a denaturing step could be used initially and could be followed by a cleavage step). Preferably cleavage is carried out enzymatically. However other means of cleavage are possible, such as chemical cleavage. (An appropriate cleavage site can be provided in said molecule). Denaturing can be performed by any suitable means. For example it may be performed by heating and / or by changing the ionic strength of a medium in the vicinity of the nucleic acid molecules." (emphasis added).

15. The first sentence in paragraph [0175] refers to the processing of double-stranded molecules for the provision of "*single-stranded molecules for use in sequencing as described above*" (emphasis added). The preceding paragraph [0174] refers in the context of a sequencing method to "*figure 17, which is entitled "in situ sequencing" "*. Since Figure 17 is silent on any sequencing, it is clear that instead Figure 15 is meant which is entitled *"in situ sequencing"*. Figure 15, however, is silent on the form of the nucleic acid to be sequenced. Figure 16(e), likewise mentioning *"in*

situ sequencing" discloses a single-stranded template wherein the second strand is completely removed.

16. Appellant II submitted that the preceding paragraphs [0151] to [0155] in document D5 described sequencing methods that included a heat denaturation step of DNA in colonies, which had the effect that both strands of a double-stranded molecule remained immobilised on the solid support (see paragraph [0155]). It is not convincing, though, that paragraphs [0151] to [0155] of document D5 directly and unambiguously describe sequencing methods. These paragraphs are headed by the title "*Preparation for hybridisation*" and belong to a section named "*(I) Nucleic acid probe hybridisation assay*", which rather relates to assays involved in the detection of immobilised molecules in colonies. However, assays for detecting nucleic acids are technically different from sequencing methods.

17. Appellant II further submitted that paragraph [0151] by referring explicitly to "*figure 16c*", which dealt with a sequencing method, provided an indication for the skilled person that paragraphs [0151] to [0155] likewise concerned methods for sequencing. Paragraph [0151] of document D5 itself is silent on any sequencing method. It describes that colonies are treated by a restriction endonuclease to generate single-stranded immobilised molecules which are required for allowing hybridisation of labelled probes for detection purposes. Figures 16(c) to (e) disclose an immobilised double-stranded molecule in bridge form that upon cutting by a restriction enzyme and a denaturation step to remove the non-attached DNA strand is converted into a single-strand. This single-strand can hybridise to a labelled probe as referred to in paragraph [0151] for detection purposes. Thus, also the

reference to Figure 16c in paragraph [0151] cannot be regarded as a direct and unambiguous disclosure of a sequencing method.

18. Paragraph [0175] of document D5 mentions that "*double stranded molecules can be used for sequencing without such processing*", i.e. without converting the immobilised molecules prior to sequencing into a single-stranded form. Two specific examples are indicated that do not require single-strands for sequencing purposes, (i) the use of a RNA polymerase and labelled ribonucleotides, and (ii) a nick translation-based method. Neither the first nor the second method use hybridised primers for sequencing, and hence do not fall within the method of claim 1.

19. Paragraph [0176] and Figure 19 of document D5 disclose methods for processing double-stranded molecules into single-strands by a cleavage of the sequences followed by denaturation. This treatment results in the complete removal of the complementary strand prior to sequencing as shown in Figure 16(e). Thus, also this paragraph and Figure 19 do not directly and unambiguously disclose that both strands of a double-stranded target remain immobilised to a solid support.

20. Paragraph [0177] discloses that the "*primers for use in sequencing preferably hybridise to the same sequences present in amplified nucleic acid molecules as do primers that were used to provide said amplified nucleic acids. (Primers having the same / similar sequences can be used for both amplification and sequencing purposes)*". In other words, PCR primers used for amplification purposes may be used for sequencing too. Paragraph [0177] starts with the formulation "*Once single-stranded molecules to be sequenced are*

provided", which implies that single-strands are sequenced in a PCR-based method. A sequencing of both complementary strands can likewise not be directly and unambiguously derived from the use of the plural form of "*primers*" in the passages cited above, since the whole paragraph refers to "*amplified nucleic acid **molecules**"* and "*single stranded **molecules** to be sequenced*" (emphasis added), i.e. various nucleic acids have been amplified, and accordingly have to be sequenced, a task that requires more than one PCR primer. Moreover, depending on the strand removed either the first or the second PCR primer may be used for sequencing.

21. A disclosure of PCR primers hybridising to complementary strands for sequencing purposes is also not derivable from paragraph [0187] that mentions the "*primer itself and its extension may be removed and replaced with another primer*" (see lines 29 and 30), but is silent on the presence of complementary strands, let alone their sequencing. Paragraph [0187] specifies various conditions for using labelled nucleotides and primer extensions in sequencing reactions, which seem to refer to the preceding paragraphs [0184] and [0186], and, hence, indicates that the primer extensions and replacements in paragraph [0187] rather concern a single-strand sequencing method (see paragraph [0184]).

22. Lastly, paragraph [0201] too does not provide a direct and unambiguous disclosure that both strands of a target polynucleotide to be sequenced remain immobilised on the solid support, since the passage is silent on this issue. It describes a re-sequencing approach in cases where the template is too long for being sequenced in a single run. Paragraph [0198] states that *de novo* sequencing and re-sequencing are

"*discussed in greater detail later on (see the following sections (v) and (vi))*". Both sections (see e.g. paragraphs [0211] and [0217]) mention explicitly the sequencing methods disclosed in the preceding "*section I(iii)*" (i.e. in paragraphs [0164] to [0201]), that, for the reasons set out above, either disclose the sequencing of double-strands by methods not falling within the claimed ambit, or if based on cycles of denaturation/hybridisation/primer extension as recited in claim 1, mention the sequencing of single-strands.

23. In light of these considerations, the opposition division's conclusions cannot be shared, because the feature that both strands of a double-stranded polynucleotide remain immobilised to a solid support throughout the sequencing process as required by claim 1 is not directly and unambiguously disclosed in document D5. Thus, claims 1 of auxiliary requests 4 and 5 comply with the requirements of Article 54 EPC.

Inventive step - claim 1

Closest prior art

24. It is disputed between the parties whether or not document D5 may be regarded as the closest prior art. Appellant I submitted that document D1 represented the closest prior art, since the sequencing method disclosed therein related to the pairwise sequencing of both strands of an immobilised target polynucleotide, a purpose identical to that of the claimed method. Contrary thereto, the method of document D5 concerned the sequencing of immobilised single-stranded polynucleotides only.

25. These arguments of appellant I are not convincing. As set out above, the method of claim 1 is directed to two alternative sequencing methods, wherein alternative A concerns the sequence determination of two separate regions on one immobilised strand only, while alternative B relates to the sequencing of two separate regions on both immobilised complementary strands. Document D5 discloses *inter alia* a method of sequencing more than one region in consecutive reactions on the same immobilised strand to determine the sequence of a long nucleic acid molecule (see paragraph [0201]), which aims at the same objective as the method of alternative A in claim 1. Moreover, it is common ground between the parties that the method of document D5 has more of the relevant technical features in common with the claimed method than the method in document D1.

26. Therefore, according to the established case law (see Case Law, I.D.3.1), document D5, and not document D1, represents the closest prior art for the method of alternative A in claim 1. This embodiment will be assessed for inventive step in the following.

27. Appellant I submitted that the method of alternative A of claim 1 differed from the method disclosed in document D5 in that the second strand was still present on the solid support, while document D5 taught the removal of the second strand. This had the advantageous effect that the skilled person had more options at hand concerning the strand to be sequenced. In light thereof, the technical problem to be solved by alternative A was considered as the provision of a more versatile pairwise sequencing method.

28. These arguments are likewise not convincing. As set out above, the method of alternative A is directed to the

sequencing of two separate regions on **one** of the two complementary strands only. The second strand is not used for sequencing, and hence, does not contribute to the technical character of alternative A of the claimed method. In other words, the second not-to-be sequenced strand is neither involved in the method of pairwise sequencing (i.e. the subject-matter of the claim), nor is it required for the sequencing of two separate regions located on the first immobilised strand (i.e. it does not form part in the solution of a technical problem). In this situation the mere presence of the second strand in alternative A cannot justify an inventive step (see Case Law, I.D.9.1).

29. Thus the technical problem to be solved by alternative A in claim 1 is the provision of an alternative pairwise sequencing method.

Obviousness

30. The technical aspect of the claimed method of alternative A, namely the pairwise sequencing of two separate regions on one strand of an immobilised double-stranded polynucleotide, is explicitly suggested in paragraph [0201] of document D5 which states in the context of re-sequencing long nucleic acid molecules that reactions are carried out in "*successive cycles applied at the same colony (where between each cycles the primers and extension products are washed off and replaced by different primers)*". In other words, alternative A of the claimed method is the consequence of using the sequencing method disclosed in paragraph [0201] of document D5. Hence, auxiliary requests 4 and 5 lack an inventive step (Article 56 EPC).

Auxiliary request 7 - claim 1

31. Auxiliary request 7 is identical to auxiliary request 7 as maintained in the first instance proceedings. Claim 1 of auxiliary request 7 differs from claim 1 of the main request in that the preamble and step (a) have been amended as in auxiliary requests 4 and 5 (see above). In addition, step (f) has been amended in that the feature "*template strand of step (c)*" was replaced by "*complement of the template strand of step (c)*", and in that the feature "*or a complement thereof*" was deleted. In other words, alternative A, as set out above, has been deleted from claim 1 which is thus restricted to alternative B, i.e. the pairwise sequencing of two separate regions on both complementary strands of an immobilised nucleic acid molecule.

Added subject-matter, extension of protection, clarity and support, novelty

32. It is uncontested that the amendments in the preamble and in step (a) of claim 1 find a basis in the patent application (see claim 1). Furthermore, contrary to appellant II's view, for the reasons set out above for claim 1 of the main request, the term "*oligonucleotides*" in steps (d) and (g) of claim 1 finds a basis in the patent application. Thus, auxiliary request 7 complies with Article 123(2) EPC.

33. Objections under Articles 123(3) and 84 EPC have not been raised by appellant II against the subject-matter of auxiliary request 7, and the board has no reason to raise any on its own motion.

34. Objections under lack of novelty based on document D5 have been raised by appellant II against the method of claim 1. Claim 1 of auxiliary request 7 is identical to the method of alternative B in claims 1 of auxiliary requests 4 and 5. Thus, for the reasons indicated above, auxiliary request 7 complies with Article 54 EPC.

35. Appellant II raised further novelty objections against the method of claim 1 based on documents D1 to D3. It submitted in this context that documents D1 to D3 were detrimental to the novelty of claim 1 for the arguments "*described in length in our submissions during opposition proceedings and the Board is referred to previously filed document for details of the arguments*" (see statement of grounds of appeal, page 6, point 3.4).

35.1 Article 12(1) RPBA 2020 states that appeal proceedings shall be based on the statement of grounds of appeal. Article 12(3) 2020 RPBA requires the statement of grounds of appeal to contain a party's complete case and to set out clearly and concisely the reasons why it is requested that the decision under appeal be reversed, amended or upheld, and should specify expressly all the requests, facts, objections, arguments and evidence relied on. According to the case law, a mere reference to submissions made at the first instance, as a rule, cannot replace an explicit account of the legal and factual reasons for the appeal (see Case Law, V.A.2.6.4., 1173 and V.A.3.2.1.j), 1195).

35.2 In line therewith, appellant II's arguments under Article 54 EPC, based on documents D1 to D3, cannot be considered.

Sufficiency of disclosure - claim 9

36. Appellant II submitted that the method according to claim 1 specified in the preamble that both strands of the double-stranded polynucleotide remained double-stranded throughout the sequencing process, and that step (f) further required that the second sequencing primer hybridised to a sequence complementary to that of the first strand. However, claim 9 which incorporated the subject-mater of claim 1 by its dependence on claim 8 was incompatible with these two requirements, because the combined action of a restriction enzyme and a denaturation step removed the complementary sequence parts of the two strands, which were, however, needed for the formation of double-strands and the hybridisation to the second sequencing primer.

37. Also these arguments are not convincing. As set out above, claim 1 neither requires that the two strands of a double-stranded polynucleotide remain immobilised in double-stranded form throughout the sequencing process, nor that the second sequencing primer hybridises to a complementary sequence of the first strand in a strict literal sense. According to claim 1, it suffices that the two complementary strands of the template to be sequenced remain immobilised to the solid support throughout the process, and that the second strand to which the second primer hybridises in step (f) is the complement of the first strand and, hence, that both strands are derived from the same immobilised double-stranded template.

38. Thus, claim 9 of auxiliary requests 4 and 5 complies with Article 83 EPC.

Inventive step - claim 1

39. The method of claim 1 is restricted to alternative B set out above, i.e. to the pairwise sequencing of two separate regions on the complementary strands of immobilised double-stranded polynucleotides.

Closest prior art

40. As set out above, document D5 is silent on the sequencing of separate regions located on both complementary strands of a double-stranded polynucleotide, i.e. the purpose underlying the method of claim 1, but rather relates to the sequencing of single-stranded nucleic acids. As regards document D1, it is common ground that it discloses a sequencing method aiming at the same purpose as the claimed method, since it mentions a method for pairwise sequencing of immobilised double-stranded nucleic acids (see document D1, page 1, lines 6 to 13, page 3, lines 3 and 4, page 4, lines 23 to 26, page 9, lines 22 and 23).

41. In line with established case law (see Case Law, I.D. 3.1), since document D1 is directed to the same purpose as the method of claim 1, while the purpose in document D5 is only similar, document D1 represents the closest prior art for the assessment of inventive step. The fact that the method of document D1 has fewer relevant technical features in common with the claimed method compared to document D5, is of less relevance in the present case.

42. Document D1 discloses a pairwise sequencing method wherein both sequencing primers are simultaneously hybridised to the single-strands of immobilised target

polynucleotides. Due to the presence of both sequencing primers at the onset of the reaction, only one primer has a free 3'-OH end ("unblocked") to start the primer extension by incorporating labelled nucleotides, while the second primer has a chemically blocked 3'-end to prevent unwanted primer extension reactions.

43. The claimed method differs from the closest prior art at least in the sequential addition of sequencing primers to the first and second sequencing reactions, and in the removal of the extended first sequencing primer generated in step (d) prior to the onset of the second sequencing reaction. It is again undisputed that these distinguishing features allow a more efficient pairwise sequencing of double-stranded polynucleotides, because it avoids the need for the chemical blocking and de-blocking of sequencing primers.

44. The technical problem can thus be defined as the provision of an improved method for pairwise sequencing of both strands of immobilised double-stranded polynucleotides.

45. It is likewise uncontested that the claimed method solves this problem.

Obviousness

46. It remains to be assessed whether or not the skilled person, in the expectation of solving the problem defined above, would have modified the teaching in document D1 in the light of the teachings of document D5 so as to arrive at the claimed method in an obvious manner.

47. Since this issue involves determining whether or not the skilled person would - and not just could - have made a particular modification, it is necessary to identify conclusive reasons on the basis of tangible evidence that would have prompted the skilled person to act in one way or the other.

48. Appellant II submitted that the skilled person had a motivation to look for improved methods of pairwise sequencing of immobilised double-stranded nucleic acids starting from the closest prior art method due to the obvious disadvantages associated with the chemical blocking and de-blocking of sequencing primers during the sequencing of both complementary strands.

49. Since it is undisputed that these chemical steps are cumbersome, the skilled person may have been motivated to look for sequencing methods that did not require the blocking and de-blocking of sequencing primers. Document D1 itself is silent on such sequencing methods, nor points at them.

50. Appellant II further submitted that the skilled person in this situation would have considered documents dealing with sequencing-by-synthesis reactions. Document D5, for example, disclosed such a reaction using sequencing primers that did not hybridise simultaneously with their target sequence, like in document D1, but sequentially after completing the first sequencing run followed by a strand denaturation to allow the second primer to hybridise. Moreover, since document D1 already disclosed a reaction cycle dealing with the blocking and de-blocking of sequencing primers, the skilled person had a high expectation of success that the replacement of this cycle by a

denaturation/hybridisation cycle allowed the sequencing of both complementary strands.

51. These arguments are also not convincing since they essentially state that the claimed method is obvious because the claimed features have been disclosed in the combined teaching of documents D1 and D5. However, the mere existence of teachings in the prior art is not a conclusive reason for explaining why the skilled person would have combined these teachings in order to solve the problem defined above. In the present case, for the reasons set out above, the denaturation/hybridisation cycle in document D5 is disclosed in the context of sequencing single-strands only, but, unlike in document D1, not for the sequencing of complementary double-strands, and hence, not for the same technical purpose. Document D5 provides also no pointers to this purpose, in particular, none can be derived from the fact that both sequencing reactions are performed in a cyclic manner, since both reactions (blocking/de-blocking and denaturation/hybridisation) are neither technically related nor mandatory for a double-stranded sequencing. An arbitrary combination of technical features designed for different purposes cannot be considered as a hint for the skilled person to arrive at the claimed method in an obvious manner.

52. Appellant II submitted further lines of argument why the combined teaching of documents D1 and D5 rendered the claimed method obvious. In that context it was stated that the sequencing of different regions on one strand provided the same information as the sequencing of both strands, and that accordingly, the selection of either one of two well-known alternative approaches was obvious, in line with decision T 1072/07 of 19 December 2008. Alternatively, it was submitted that

the claimed method was rendered obvious by the suggested use of PCR amplification primers for sequencing in document D5 when combined with the closest prior art method.

53. However, all these attempts must fail, since, as set out above, document D5 neither discloses nor suggests the use of a reaction cycle comprising a strand denaturation/sequential primer hybridisation step for the sequencing of complementary double-strands. Thus, the skilled person, contrary to appellant II's view, would not have considered the teaching of document D5 as relevant for solving the problem defined above, and would not have used it to modify the closest prior art method. Moreover, the situation in the present case differs fundamentally from that in decision T 1072/07. In that case the skilled person had to make merely a choice between two well known suggested possibilities, i.e. the use of air-gas or oxygen-gas type burners to solve the technical problem (the finding of a suitable burner), while in the present case the sequential addition of primers in combination with a denaturation/ hybridisation cycle for the sequencing of both strands of an immobilised template polynucleotide molecule was not suggested in document D5.

54. Thus, the claimed method cannot be considered obvious and auxiliary request 7 complies with Article 56 EPC.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated