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Datasheet for the decision of 18 June 2024

Case Number: T 1362/21 - 3.3.08

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Language of the proceedings: EN

Title of invention:
Sequencing methods

Patent Proprietor:
Illumina, Inc.

Opponent:
Kilger, Christian

Headword:
Sequencing methods/ILLUMINA

Relevant legal provisions:
EPC Art. 84, 54, 56, 83, 123(2), 123(3)
RPBA 2020 Art. 13(2)

Keyword:
Main request - clarity - (no)
Main request A and main request 2B - admittance into the proceedings - (no)
Auxiliary request 1 - requirements of the EPC fullfilled - (yes)

Decisions cited:
G 0010/91, G 0003/14, T 1063/92, T 0153/93, T 0449/15, T 2172/15, T 0032/16

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Case Number: T 1362/21 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 18 June 2024

Appellant: (Opponent)	Kilger, Christian Wachtelstr. 4 14195 Berlin (DE)
Representative:	Kilger, Christian CH Kilger Anwaltspartnerschaft mbB Fasanenstraße 29 10719 Berlin (DE)
Respondent: (Patent Proprietor)	Illumina, Inc. 5200 Illumina Way San Diego, CA 92122 (US)
Representative:	Tollervey, Rebecca Mewburn Ellis LLP Aurora Building Counterslip Bristol BS1 6BX (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 23 July 2021 concerning maintenance of the European Patent No. 2427572 in amended form**

Composition of the Board:

Chair	T. Sommerfeld
Members:	M. Montrone
	A. Bacchin

Summary of Facts and Submissions

I. An appeal was lodged by the opponent ("appellant") against the decision of an opposition division according to which the European patent No. 2 427 572 could be maintained in amended form. This patent is based on European patent application No. 10 770 456.1 which has been filed as International patent application published as WO 2010/127304 (the "patent application").

II. A first decision of an opposition division to reject the opposition and to maintain the patent as granted was set aside in appeal due to a lack of sufficiency of disclosure (T 2172/15). The case was remitted to the opposition division for further prosecution on the basis of auxiliary requests 1 to 8 submitted with the letter dated 7 July 2016.

III. During these opposition proceedings, the patent proprietor ("respondent") withdrew auxiliary requests 1 and 5 with letter dated 25 February 2021 and made previous auxiliary request 2 their new main request. Moreover, previous auxiliary requests 3, 4, 6, 7 and 8 were maintained and renumbered as auxiliary requests 1 to 5, respectively.

IV. In the present decision under appeal, the opposition division held that the main request complied with the requirements of the EPC.

V. With their statement of grounds of appeal, the appellant submitted arguments against the subject-matter of claims 1 and 11 of the main request under added subject-matter (Article 123(2) EPC), lack of

clarity (Article 84 EPC), sufficiency of disclosure (Article 83 EPC) and under lack of novelty and inventive step (Articles 54 and 56 EPC).

VI. In reply, the respondent *inter alia* re-submitted auxiliary request 1 and counter arguments.

VII. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's preliminary opinion.

VIII. In response, the respondent submitted *inter alia* a new main request A and a further document while the appellant withdrew their request for oral proceedings.

IX. Oral proceedings were held in the format of a videoconference as requested by the respondent in the absence of the duly summoned appellant, as announced. During the oral proceedings, the respondent submitted new main request 2B.

X. Claim 1 of the main request reads:

1. A method for obtaining nucleic acid sequence information, said method comprising performing iterations of:

(a) at least one dark extension step comprising providing a dark extension sequencing reagent to a target nucleic acid in the presence of a polymerase, said dark extension sequencing reagent comprising one or more nucleotide monomers wherein said nucleotide monomers are selected from non-promiscuous nucleotide monomers which each pair with one nucleotide type in said target and promiscuous nucleotide monomers which each pair with two nucleotide types in said target,

wherein together the nucleotide monomers in the dark extension sequencing reaction pair with at least two nucleotide types in said target and with no more than three nucleotide types in said target, thereby forming a polynucleotide complementary to at least a portion of said target; and

(b) at least one read extension step comprising providing a read extension sequencing reagent to said target nucleic acid in the presence of a polymerase, said read extension sequencing reagent comprising at least one nucleotide monomer, said at least one nucleotide monomer of said read extension sequencing reagent comprising a reversibly terminating moiety, the read extension step further comprising removing unincorporated read extension sequencing reagent, detecting incorporation of the at least one nucleotide monomer of said read extension sequencing reagent into said polynucleotide, and removing said reversibly terminating moiety;

wherein (a) and (b) can be carried out in either order;

whereby sequence information is obtained comprising regions in which single nucleotide assignments are not made interspersed by regions comprising at least two consecutive positions that are assigned with single base resolution.

XI. Claim 1 of main request A differs from claim 1 of the main request in that in step (a) the feature "*wherein together the nucleotide monomers*" has been replaced by "*wherein the set of one or more nucleotide monomers*".

XII. Claim 1 of main request 2B differs from claim 1 of the main request in that in step (a) the feature "*wherein*

together the nucleotide monomers in the dark extension sequencing reaction pair" has been replaced by "*wherein said dark extension sequencing reagent pairs*".

XIII. Claim 1 of auxiliary request 1 differs from claim 1 of the main request in that step (a) has been replaced by "*at least one dark extension step comprising providing a dark extension sequencing reagent to a target nucleic acid in the presence of a polymerase, said dark extension sequencing reagent comprising two or more different nucleotide monomers, wherein said two or more nucleotide monomers pair with at least two nucleotide types in said target and with no more than three nucleotide types in said target, thereby forming a polynucleotide complementary to at least a portion of said target; ...*".

Claim 10 of auxiliary request 1 reads:

"10. A method for obtaining nucleic acid sequence information, said method comprising performing iterations of:

(a) at least one dark extension step comprising providing a dark extension sequencing reagent to a target nucleic acid in the presence of a polymerase, said dark extension sequencing reagent comprising a plurality of different nucleotide monomers, wherein at least one nucleotide monomer of said plurality of nucleotide monomers comprises a reversibly terminating moiety, thereby forming a polynucleotide complementary to at least a portion of said target, and said dark extension step further comprising removing the reversibly terminating moiety of said at least one monomer of said dark extension sequencing reagent; and

(b) at least one read extension step comprising providing a read extension sequencing reagent to said target nucleic acid in the presence of a polymerase, said read extension sequencing reagent comprising at least one nucleotide monomer, said at least one nucleotide monomer of said read extension sequencing reagent comprising a reversibly terminating moiety, the read extension step further comprising removing unincorporated read extension sequencing reagent, detecting incorporation of the at least one nucleotide monomer of said read extension sequencing reagent into said polynucleotide, and removing said reversibly terminating moiety;

wherein (a) and (b) can be carried out in either order;

whereby sequence information for at least a portion of said target nucleic acid is obtained comprising regions in which single nucleotide assignments are not made interspersed by regions comprising at least two consecutive positions that are assigned with single base resolution".

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

D4: WO 2009/054922

D5: WO 2009/051807

XV. The appellant's written submissions, insofar as relevant to the present decision, may be summarised as follows:

Main request

Clarity

The amendments introduced in step (a) of claim 1 rendered the claim unclear. Reference in this context was made to "***monomers which each pair with two nucleotide types in said target,*** *wherein* ~~*said one or more*~~ *together the nucleotide monomers* ***in the dark extension sequencing reaction*** *pair with at least two nucleotide types in*". The meaning of "*together these nucleotides pair with 2 not 3*" was held unclear.

Auxiliary request 1

Added subject-matter

The feature "*detecting incorporation of the at least one nucleotide monomer of said read extension sequencing reagent into said polynucleotide*" in claim 11 of the main request (being present in claims 1 and 10 of auxiliary request 1 too) required a "*detection during incorporation*". Such a detection had no basis in paragraph [0157] of the application as filed and comprised added subject-matter.

Clarity

Further the introduction of the feature "*detecting incorporation of the at least one nucleotide monomer of said read extension sequencing reagent into said polynucleotide*" in claim 11 of the main request (being present in claims 1 and 10 of auxiliary request 1 too) led to a lack of clarity. This feature required as a minimum that one nucleotide was read which was inconsistent with the additional requirement in claim 11 that at least two consecutive nucleotides were read.

Sufficiency of disclosure

The reagent as defined in step (a) of claim 1 of the main request comprised as embodiment a single non-promiscuous nucleotide. Since this nucleotide was unable to pair with two nucleotides as likewise functionally required by step (a) of claim 1, this embodiment was a non-working one.

Also claim 11 of the main request comprised a non-working embodiment. This embodiment concerned the use of one read extension reaction in step (b) which implied that the identity of only one nucleotide was determined. However this was incompatible with the further requirement in step (b) that at least the identity of nucleotides at "*two consecutive positions*" had to be determined.

Novelty

The methods as defined in claims 1 and 11 of the main request lacked novelty over the disclosure of either documents D4 or D5, which shared a "*similar content*". In particular, the disclosure of Figures 13 and 27 of document D4 anticipated the subject-matter of the two claimed methods. Similar considerations applied for Figures 28 and 40 of document D5. Since the methods of the invention were directed solely to obtaining sequence information, this information was disclosed in some of the methods disclosed in documents D4 and D5 too.

Inventive step

Document D4 represented the closest prior art. Figure 30 in conjunction with "*Method 1*" on pages 80 and 81 of

document D4 disclosed a sequence that comprised segments of assigned nucleotides interspersed by segments of unassigned nucleotides. The method as disclosed in Figure 30 was thus identical to that of claim 1. There was also a reasonable expectation that based on the teaching of document D4 primers were extended by dark extension steps at the end of a series of read cycles for accomplishing complete sequences. Document D4 disclosed many examples relating to the combination of labelled and unlabelled terminators.

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

Main request

Clarity

The subject-matter of step (a) of claim 1 was clear. The concept of a dark extension was disclosed in paragraphs [0142] and [0143] of the application as filed. Step (a) of claim 1 encompassed various embodiments by specifying that "*one or more nucleotide monomers*" were used in the reagent "*wherein together*" these monomers "*pair with at least two nucleotide types in said target with no more than three nucleotide types in said target*". Since the meaning of a dark extension reaction was clear to the skilled person, a potential inconsistent or mutual exclusive meaning of the terms "*one*" and "*together*" in step (a) of claim 1 was not the sole sensible interpretation of the claim. The term "*together*" meant "*in combination*" or "*collectively*". Since step (a) of claim 1 encompassed various nucleotide embodiments, an interpretation of the term "*together*" without disregarding the explicit mentioning of "*one*" in step (a) related to the collective pairing

properties of the nucleotides in the reagent. In other words, the monomers paired collectively with at least two monomers but no more than three monomers in the target sequence irrespective of the amount of monomers present in the reagent.

Admittance/consideration in the proceedings of main request A and main request 2B

Main request A was submitted in direct response to the board's preliminary opinion which for the first time addressed an issue under lack of clarity. Although the appellant had raised an objection under lack of clarity, this objection was not substantiated. The present situation was thus similar to that in T 32/16. Main request A was thus filed in time. An objection against the request's admittance had not been submitted by the appellant.
Moreover, the amendment in step (a) of claim 1 (see section XI above) had a basis in paragraph [0142] of the application as filed, although the term "*set*" was not explicitly mentioned therein. This amendment did not introduce new information when compared to paragraph [0142] of the application as filed. Further the term "*set*" did not necessarily imply that more than one nucleotide was present in the dark extension reagent of step (a) in claim 1. This was illustrated by a comparison between the terms "*set*" and a "box". A hat in a box had a different meaning than a hat alone without however requiring that the box contained at least two hats. In fact a box could contain any number of hats. Thus the term "*set*" in step (a) related to any number of nucleotides.

As regards main request 2B, its submission represented the first opportunity to address an objection under

lack of clarity raised in the board's preliminary opinion. The amendment in claim 1 addressed a further potential issue under added subject-matter since it was literally disclosed in paragraph [0142] of the application as filed. Thereby no new issues were introduced, in particular the assessment of sufficiency, novelty and inventive step remained the same as for the main request. The late filing of main request 2B did not affect any third parties either, since the appellant had deliberately decided not to attend the oral proceedings.

Auxiliary request 1

Added subject-matter

The subject-matter of claim 1 had a basis in claims 3 and 4 as filed in conjunction with paragraphs [0008] and [0009] of the application as filed.

Admittance of a new line of argument under added subject-matter

The appellant's line of argument under added subject-matter against claim 11 of the main request (i.e. claim 10 of auxiliary request 1) was new in the proceedings and should not be admitted.

Clarity

The appellant's objection under lack of clarity against claim 11 of the main request was not to be considered in view of decision G 3/14 since the alleged inconsistency was already present in claim 12 as granted and not caused by the amendment.

Sufficiency of disclosure

The method of claim 1, by explicitly requiring that at least two nucleotides paired with two or three nucleotides in the target sequence, excluded from step (a) the non-working embodiments that either one of the nucleotides paired with three different nucleotides (single promiscuous nucleotide) or with two nucleotides (single non-promiscuous nucleotide). The method of claim 1 was thus sufficiently disclosed in the patent application.

Admittance of an argument under insufficiency which was not submitted during the appellant's first statement of grounds of appeal

The appellant's objection under insufficiency against read step (b) in claim 11 of the main request did not form part of the appeal case T 2172/15, nor was it raised during the second opposition proceedings before the opposition division. There was no justification for this objection being raised now in appeal. Accordingly, this line of argument should be disregarded.

Novelty

The methods of claims 1 and 10 were novel over the disclosure of documents D4 and D5. The methods disclosed in document D4 and D5 provided the complete sequence information of the target sequence and not a sequence "*comprising regions in which single nucleotide assignments are not made interspersed by regions comprising at least two consecutive positions that are assigned with single base resolution*". Furthermore, the method as disclosed in Figure 27 disclosed solely one region with non-assigned nucleotides while the claimed

method required that the sequence information obtained comprised "*regions*" of non-assigned nucleotides, i.e. information from more than one region.

Inventive step

The appellant in essence argued that there was no difference between the methods of claims 1 and 10 and that disclosed in document D4. This was wrong. The methods of claims 1 and 10 produced a "bar code"-like sequence comprising regions wherein at least two consecutive nucleotides were determined interspersed with regions without nucleotide assignments. Document D4 was directed to a different purpose, i.e. the determination of complete sequence information of a target sequence. The technical problem to be solved was thus the provision of a different method of obtaining sequencing information. Since the sequence information obtained by the claimed methods was not complete, the skilled person starting from the method of document D4 had no pointer to modify said method such as to arrive in an obvious manner at the methods of claims 1 or 10.

XVII. The appellant requested in writing that the decision of the opposition division be set aside and that the patent be revoked. The appellant moreover requested a reimbursement of the appeal fee.

XVIII. The respondent requested that the appeal be dismissed (main request) or, alternatively that the patent be maintained on the basis of the claims of one of main request A, main request 2B, or auxiliary requests 1, 2, 2A, 3, 3A, 4, 5 or 5A, being that auxiliary requests 1 to 5 have been filed with the reply to the appeal while main request A and auxiliary requests 2A, 3A and 5A have been filed with letter 3 May 2024 and main request

2B has been filed at the oral proceedings in appeal. Moreover, the respondent requested that new arguments under added subject-matter and insufficiency not be admitted and that the appellant's request for reimbursement of the appeal fee be rejected.

Reasons for the Decision

Main request

Claim construction - claim 1

1. Claim 1 is directed to a "*method for obtaining nucleic acid sequence information, said method comprising performing iterations of:*

> *(a) at least one dark extension step comprising (...); and*
>
> *(b) at least one read extension step comprising (...);*
> *wherein (a) and (b) can be carried out in either order;*
> *whereby sequence information is obtained comprising regions in which single nucleotide assignments are not made interspersed by regions comprising at least two consecutive positions that are assigned with single base resolution*".

2. Thus the method of claim 1 concerns a method for obtaining sequence information of any nucleic acid type (e.g. DNA or RNA) which comprises iterations of at least two process steps in any order:
(a) "*one dark extension step*" and
(b) "*one read extension step*".

3. The feature "*dark extension*" in claim 1 relates to a limited extension of a polynucleotide complementary to a target sequence through a polymerase due to the lack of at least one nucleotide monomer. The patent discloses that "*one purpose of this process is to extend down a target nucleic acid without necessarily reading the sequence of the target nucleic acid*" (paragraphs [0135] and [0136]). Thus during a dark extension as defined in step (a) of claim 1 a nucleic acid sequence may be extended by a polymerase without being necessarily sequenced, i.e. the target's sequence information remains in the "dark".

3.1 Step (a) of claim 1 achieves this purpose by providing a "*dark extension sequencing reagent*" to a target nucleic acid in the "*presence of a polymerase*", said dark extension sequencing reagent comprising "*one or more nucleotide monomers*". These monomers are further structurally and functionally defined in that they are "*selected from non-promiscuous nucleotide monomers which each pair with one nucleotide type in said target and promiscuous nucleotide monomers which each pair with two nucleotide types in said target*". Furthermore since the dark extension step is polymerase-based it is implicit that an extension primer must be present too.

3.2 The board agrees with the opposition division (decision under appeal, points 5.3 and 5.4) that the requirement that the "*one or more monomers*" in step (a) of claim 1 have to be "*selected from non-promiscuous*" (emphasis added) and "*promiscuous*" nucleotide monomers limits the dark extension reagent to the use of at least one nucleotide selected from two alternative categories:

- a "*non-promiscuous*" nucleotide (i.e. a standard nucleotide: A, T, C, G, or U) that pairs with one nucleotide type in the target sequence and

- a "*promiscuous*" nucleotide that pairs with two nucleotide types of the target sequence.

This limitation excludes from claim 1 the presence of the non-working embodiment concerning the use of one nucleotide that pairs with three different nucleotide types (T 2172/15, Reasons 4.1 to 4.3 and 5).

3.3 Moreover, step (a) requires functionally that "*wherein together the nucleotide monomers in the dark extension sequencing reaction pair with at least two nucleotide types in said target and with no more than three nucleotide types in said target, thereby forming a polynucleotide complementary to at least a portion of said target*" (emphasis added). In other words, step (a) requires that the "*one or more*" nucleotides selected from "*non-promiscuous*" and "*promiscuous*" monomers pair "*together*" (i.e. in combination) with two or three target nucleotide types.

4. The term "*read extension*" in step (b) of claim 1 is widely used in the field and refers to a polymerase-based sequencing for obtaining sequence information from a target sequence.

5. The appellant contested that the functional feature indicated in the last part of claims 1 and 11 which relates to a result to be achieved ("*whereby sequence information [for at least part of said target nucleic acid: claim 11] is obtained comprising regions in which single nucleotide assignments are not made interspersed by regions comprising at least two consecutive positions that are assigned with single base resolution*") limits the claimed methods.

5.1 This functional feature is the result of combining at least one dark and one read extension reaction in any

order as defined in steps (a) and (b) of claims 1 and 11. Thereby gapped sequence information is obtained which comprises regions with at least two consecutive identified nucleotides ("*assigned*") interspersed by regions containing an undefined number of non-identified nucleotides ("*assignments are not made*").

5.2 The appellant stated that this functional feature "*cannot be considered a technical feature, as it is a result to be achieved and in the present wording has no technical limiting effect on the claim*" (statement of grounds of appeal, page 9, second paragraph) without providing reasons for this assertion.

5.3 The board does not agree. Functional features defined by a result to be achieved are not necessarily non-limiting (Case Law of the Boards of Appeal, 10th edition 2022 ("Case Law"), II.A.3.4). Since claims 1 and 11 are not directed to the preparation of a product but to a method for obtaining information on a property of nucleic acids, i.e. their sequence information, this functional feature limits the claimed methods (Case Law, I.C.8.1.3 c)).

Clarity - claim 1

6. It was a matter of dispute whether or not the terms "*one or more nucleotide monomers*" and "*together*" in step (a) of claim 1 introduced an ambiguity which rendered the claim unclear.

7. The respondent and the opposition division were of the view that the term "*together*" in the context of step (a) of claim 1 referred to the collective pairing properties of the nucleotide monomers in the sequencing reagent as a whole. The respondent further submitted

that the skilled person would not give "*together*" a meaning that would override the explicit mentioning of "*one or more nucleotide monomers*" (emphasis added).

8. The board does not agree. The term "*together*" in step (a) of claim 1 according to its ordinary meaning implies that the sequencing reagent comprises at least two nucleotide monomers for a pairing with either two or three different nucleotide types in the target sequence. The wording of claim 1 further implies that "*together*" refers to all monomers in the reagent.

8.1 Furthermore, step (a) of claim 1 requires that at least one nucleotide monomer ("*one or more*") is used in the reagent. Consequently, the reagent of step (a) comprises the following separate embodiments (as regards the meaning of promiscuous and non-promiscuous see point 3.2 above):

- a reagent with a single promiscuous nucleotide
- a reagent with either two or three non-promiscuous nucleotides
- a reagent with a combination of a single non-promiscuous nucleotide and a single promiscuous nucleotide.

8.2 However, the terms "*together*" and "*one*" monomer as used in step (a) of claim 1 have a mutually exclusive meaning. That this is a technically sensible construction of claim 1 is uncontested. The respondent contested rather that this was the sole sensible construction of these terms in step (a) as a construction was likewise sensible which related to the collective pairing properties of the nucleotides in the sequencing reagent as a whole without disregarding the explicit mentioning of using one nucleotide only.

8.3 Since it is uncontested that several technically sensible interpretations of step (a) in claim 1 exist, the skilled person faced with this situation is left in doubt which of them is the correct one and in particular, whether or not a reagent with a single promiscuous nucleotide (point 8.1 above) falls within the scope of claim 1 or not. An explicit mentioning of at least "*one*" monomer is of no help in this case since for the reasons set out above claim 1 allows more than one sensible claim interpretation.

9. The subject-matter of claim 1 thus lacks clarity contrary to the requirements of Article 84 EPC.

Main request A and main request 2B

10. Claim 1 of main request A has been amended in essence when compared to claim 1 of the main request in that the term "*together*" in step (a) has been replaced by "*the set of one or more*".

11. Claim 1 of main request 2B has been amended compared to claim 1 of the main request in that in step (a) the feature "*together the nucleotide monomers in the dark extension sequencing reaction pair*" has been replaced by "*said dark extension sequencing reagent pairs*".

Admittance/consideration in the proceedings of main request A and main request 2B

12. Main request A has been submitted by the respondent in response to the communication setting out the board's preliminary opinion. Main request 2B has been submitted by the respondent at the oral proceedings only.

13. According to Article 13(2) RPBA the submission of these two new requests represents an amendment of the respondent's appeal case which is not to be taken into account unless there are exceptional circumstances justified by cogent reasons.

14. As regards main request A, the respondent argued in support of its admittance that this set of claims was filed in response to a new objection under Article 84 EPC raised for the first time by the board in its preliminary opinion. Although the appellant raised an objection under Article 84 EPC against step (a) of claim 1, this objection was never substantiated, neither during opposition proceedings, in particular with the submission of 10 February 2021, nor at the appeal proceedings. In addition, the appellant had not objected to the admittance of main request A.

15. The provision of Article 13(2) RPBA *inter alia* requires the party, in this case the respondent, to provide reasons for submitting the amendment at this stage of the appeal proceedings. In exercising its discretion, the board has to consider also whether the party has demonstrated that the amendment *prima facie* overcomes the issues raised by the appellant or by the board and does not give rise to new objections.

16. The board is not convinced that there is a justification for filing main request A at such a late stage in the proceedings. In particular, it does not share the respondent's view that the filing of the present amendment was justified by the board's preliminary opinion expressed in the communication under Article 15(1) RPBA.

17. The clarity objection had been on file since the opposition proceedings were continued after remittal from the first board of appeal's decision (appellant's submissions of 10 February 2021, pages 14 to 16).

17.1 The fact that the clarity objection consisted in an intrinsic contradiction of the terms "*together*" and "*one*" monomer is apparent already from the appellant's reproduction of the plain wording of the claim and the highlighting of the terms "*monomers*" and "*together*" as an indication that such wording creates an ambiguity that renders the claim unclear (pages 12 and 16 of the statement of grounds of appeal).

17.2 The opposition division was able to understand the essence of the objection, since at point 4.1 of the reasons of the appealed decision it is indicated: "*The opponent argues that the new features added to claim 1 introduce a lack of clarity because "it is not clear what is meant" by the 'together' expression*." The objection was also understandable, as the opposition division was in the position to provide a reasoning for its finding that the claimed subject-matter was clear. The fact that the opposition division understood the expression as referring to the collective pairing capabilities of the nucleotide mixture in the dark sequencing reagents does not exclude that the amended claim is also open to further interpretations. In particular, it does not exclude the ambiguity pointed out by the appellant (point 17.1, above).

17.3 The board is not convinced that the fact that the opposition division found the requirements of Article 84 EPC to be met by the claimed subject-matter constituted a reason for the respondent not filing an appropriate fall-back position with its reply to the

statement of grounds of appeal. On the contrary, the respondent had reasons to file the present amendment at the latest with the reply to the statement of grounds of appeal.

17.4 The considerations made for instance in case T 32/16, to which the respondent referred in the course of the oral proceedings before the board, are not applicable to the present situation. In that case the board's communication issued under Article 15(1) RPBA did "*crystallise*" for the first time what the board itself had deduced to be the relevant elements of the appellant's objections (T 32/16, Reasons 1.3), which *de facto* amounted to raise a new objection. In the present case, it cannot be said that the board raised a new objection under Article 84 EPC, suitable to justify the filing of new amended claims within the meaning of Article 13(2) RPBA. Rather the board explicitly formulated the objection that was apparent from the emphasis given by the appellant for the respective terms (point 17.1 above) which indicated in essence that the terms "*together*" and (one) "*monomer*" according to step (a) of claim 1 have a mutually exclusive meaning. Indeed the term "*together*" in step (a) of claim 1 according to its ordinary meaning implies that the sequencing reagent comprises at least two nucleotide monomers for a pairing with either two or three different nucleotide types in the target sequence.

17.5 That the board's communication did not raise a new objection is also apparent from the respondent's submissions. In particular, the respondent's line of defence in support of clarity of claim 1 of the main request, which was provided with the submissions of

3 May 2024, after the board's communication under Article 15(1) RPBA, has substantially remained the same to that provided in the reply to the statement of grounds of appeal. It essentially relies on an understanding of the term "*together*" as referring to the collective properties of the nucleotide monomers in the reagent, as contrasted with the individual properties of those reagents.

17.6 Therefore no justification can be found in the content of the board's communication under Article 15(1) RPBA for the filing of the present main request A.

17.7 As to the further considerations for the board's exercise of discretion, namely whether the party has demonstrated that the amendment *prima facie* overcomes the issues raised by the appellant and does not give rise to new objections, the following is found.

17.8 First, the board is not persuaded that the amendment introduced in main request A, by removing the word "*together*" and replacing it by the wording "*...wherein the set of one or more nucleotide monomers...*" *prima facie* overcomes the clarity objection at stake.
The respondent argued that the term "*set*" had to be understood as a "box". This term did not necessarily require that the box contained at least two items, but could contain either no item (an empty set), which however did not make sense in the present context, or one item, or a plurality of items. Thus the term "*set*" in step (a) had to be understood as being related to any number of nucleotides, including one nucleotide, so that the ambiguity issue raised by the appellant and followed by the board was removed.

17.9 The board does not share the view that the wording "*set of one or more nucleotide monomers*" is to be given the meaning submitted by the respondent, namely to include one nucleotide for a pairing with either two or three different nucleotide types in the target sequence. Claim 1 of main request A still allows more than one sensible technical interpretation, in particular it still includes that the reagent may comprise at least two nucleotide monomers for a pairing with either two or three different nucleotide types in the target sequence, in contradiction with the explicit mentioning of "*one or more nucleotide monomers*".

17.10 Second, the board is also not convinced that the amendment does not raise further objections. In particular, the board fails to see a basis for the amendment in paragraph [0142] of the application as filed. As it was also acknowledged by the respondent in the submissions of 3 May 2024 (page 3, penultimate paragraph) and at the oral proceedings before the board, no mention at all is made in that passage of the term "*set*", nor can it be considered as implicitly disclosed from the whole of the application and certainly it is not disclosed in a direct and unambiguous manner. In this context and in order to address the respondent's submission that an objection of added subject-matter had not been raised by the appellant against the main request, the board underlines that it is an established principle under the EPC that in case of amendments of the claims or other parts of a patent in the course of opposition or appeal proceedings, such amendments are to be fully examined as to their compatibility with the requirements of the EPC, e.g. with regard to the provisions of Article 123(2) and (3) EPC (G 10/91, OJ 1993, 420, Reasons 19).

18. As regards main request 2B, the respondent in essence justified the late submission of this request by stating that this set of claims was *prima facie* allowable: it addressed both the clarity issues raised for the first time in the board's communication and further addressed a potential issue under added subject-matter, as the term "*sequencing reagent*" was literally disclosed in paragraph [0142] of the application as filed. Since the amendment was merely intended to find a proper wording, the questions of sufficiency, novelty and inventive step remained the same as for the main request.

19. The board considers that the same reasons provided above for main request A, regarding the lack of a justification for filing amendments addressing the clarity objection in reaction to the board's communication, apply also to main request 2B. In addition, the board finds that the present amendment is even far more reaching than the one of main request A and *prima facie* raises additional issues, for instance, that it is unclear how the "*dark extension reagent*" might pair with the nucleotide types indicated in step (a) of claim 1. Nor is it apparent which impact this amendment has on the question of novelty, inventive step and sufficiency which the board would have to assess anew at the oral proceedings. The board therefore does not agree with the respondent that the amendment, while aiming at finding a proper wording in avoiding issues under added subject-matter, leaves the questions of sufficiency, novelty and inventive step the same as for the main request. Its admittance at this stage would thus be detrimental to procedural efficiency.

20. In view of these considerations, the board decided not to admit any of main request A and main request 2B (Article 13(2) RPBA).

Auxiliary request 1

21. Claim 1 of auxiliary request 1 differs in essence from claim 1 of the main request in that the sequencing reagent in step (a) has been limited to comprise "*two or more different nucleotide monomers*" instead of "*one or more nucleotide monomers*". Claim 10 of auxiliary request 1 is identical to claim 11 of the main request.

Claim construction

22. The dark extension sequencing reagent of claim 1 when compared to claim 1 of the main request is thus limited to comprise at least two different monomers which are not further defined except that these monomers must "*pair with at least two nucleotide types in said target and with no more than three nucleotide types*".

23. Step (a) of claim 1 encompasses thus as separate embodiments:
- a reagent with either two or three different non-promiscuous nucleotides (i.e. standard nucleotides selected from A, T, C, G, or U that pair each with one nucleotide type in the target sequence only), or
- a reagent with a combination of a single non-promiscuous nucleotide and a single promiscuous nucleotide (i.e. a nucleotide that pairs with two nucleotide types of the target sequence).

24. Since step (a) of claim 1 is limited to a dark extension reagent that comprises at least two different

monomers, claim 1 excludes the non-working embodiments relating to the use of a single promiscuous nucleotide that pairs with three different nucleotide types (T 2172/15, Reasons 4.1 to 4.3 and 5) and the use of a single non-promiscuous nucleotide that pairs with two nucleotide types in a target sequence. The appellant's objection submitted under sufficiency of disclosure against claim 1 of the main request as regards the latter embodiment is thus rendered moot.

25. Claim 10 relates to a further independent method for obtaining nucleic acid sequence information.

25.1 The method of claim 10 defines the dark extension sequencing reagent in step (a) as comprising "*a plurality of different nucleotide monomers*" wherein "*at least one*" of these monomers "*comprises a reversibly terminating moiety*". The pairing properties of these monomers are not further specified in claim 10. Since the ordinary meaning of "*plurality*" is two or more, the reagent as defined in step (a) of claim 10 comprises at least two different nucleotides of any type with undefined pairing properties of which at least one contains a reversible terminator.

25.2 Contrary thereto, the reagent as defined in step (a) of claim 1 comprises at least two nucleotides lacking a reversible terminator.

Added subject-matter

26. In the following references to the claims as filed or application as filed are to the patent application (WO 2010/127304).

Admittance of a new line of argument under added subject-matter

27. The appellant submitted in their statement of grounds of appeal that the feature "*detecting incorporation of the at least one nucleotide monomer of said read extension sequencing reagent into said polynucleotide*" in claim 11 of the main request (i.e. claim 10 of auxiliary request 1) required a "*detection during incorporation*" which had no basis in paragraph [0157] of the application as filed and hence comprised added subject-matter.

28. The question thus arises whether or not the appellant's submission under added subject-matter represents an amendment of their case which may be admitted only at the discretion of the board (Article 12(4) RPBA).

29. The contested feature in claim 10 (which is present in claim 1 too) has been introduced into all auxiliary requests submitted on 24 August 2015, i.e. during the first opposition proceedings. This includes auxiliary request 3 which is identical to present auxiliary request 1 (see section III above). The patent proprietor indicated as basis for this feature paragraphs [0157], [0165], [0166] and [0180] of the application as filed (letter dated 24 August 2015, page 6, paragraphs following the heading "*First Auxiliary Request*").

30. At the second opposition proceedings, the appellant stated in their reply to the summons dated 10 February 2021 on page 17, second paragraph that "*Concerning the amendment of claim 11 of Auxiliary Request 2 patentee provides no basis at all in the letter of 2015 as to where in the application as filed the newly added features may be found*" (auxiliary

request 2 mentioned here being identical to the present main request). This assertion is however wrong, as is apparent from the previous paragraph.

31. In view of this course of events, the board agrees with the respondent that the appellant's argument under added subject-matter against claim 11 of the main request (identical to claim 10 of auxiliary request 1) is new to the proceedings and represents an amendment of the appellant's case (Article 12(4) RPBA). Since this amendment in claim 11 of the main request or in present claim 10 is on file since the first opposition proceedings, the appellant's objection should have been raised earlier and is therefore disregarded in the proceedings (Article 12(4) RPBA).

32. The appellant submitted that the feature "*detecting incorporation of the at least one nucleotide monomer of said read extension sequencing reagent into said polynucleotide*" in claims 1 and 10 required a "*detection during incorporation*" and thus comprised added subject-matter. This is not persuasive since contrary to appellant's view, claims 1 and 11 do not require any such detection during incorporation but after incorporation. The method of claims 1 and 10 has thus a basis on claims 3 and 4 as filed in conjunction with paragraphs [0008], [0009], [0157] and [0165] of the patent application.

32.1 Auxiliary request 1 meets therefore the requirements of Article 123(2) EPC.

Extension of protection and clarity

33. Furthermore since the method of claim 1 has been limited compared to the method of claim 1 as granted

due to the use of "*two or more different nucleotide monomers*" in step (a) instead of "*one or more nucleotide monomers*", auxiliary request 1 meets the requirements of Article 123(3) EPC.

34. The method of claim 1 is further devoid of inconsistencies and ambiguities.

34.1 The appellant's objection against claim 11 of the main request (identical to present claim 10) under lack of clarity is not admissible in view of G 3/14 (headnote).

34.2 In line with the opposition division's decision (Reasons, point 4.2) the inconsistency referred to by the appellant is not caused by the amendment. Claim 12 as granted, on which in essence present claim 10 is based, mentions in step (b) "*at least one read extension step*" and at the end of the claim a read "*comprising at least two consecutive positions*". Thus, any inconsistency between step (b) of claim 11 of the main request or present claim 10 concerning one read extension step and a read at two consecutive positions was already present in granted claim 12. While the appellant reiterated this lack of clarity objection in their statement of grounds of appeal, the appellant did not submit any reasoning why the opposition division's finding was erroneous that this objection could not be considered in view of G 3/14.

35. Auxiliary request 1 therefore complies with Article 84 EPC.

Sufficiency of disclosure - claims 1 and 10

36. The appellant submitted that the invention as defined in claim 1 was not disclosed in the patent application

in a manner sufficiently clear and complete for it to be carried out by the skilled person (Article 83 EPC).

37. The board does not agree. In particular, combinations of at least two different nucleotide monomers are available to the skilled person which allow a pairing with either two or three different nucleotide types as functionally required in claim 1 (point 23 above).

Admission of an argument under insufficiency which was not submitted during the appellant's first statement of grounds of appeal

38. The appellant submitted that the method of claim 11 of the main request (identical to present claim 10) was insufficiently disclosed. The read extension step (b) of claim 11 required in one embodiment the presence of one terminating nucleotide which allowed the determination of one nucleotide. This was incompatible with the additional need for determining "*at least two consecutive positions*".

39. The respondent requested to disregard this objection since it did not form part of the appellant's first appeal and the present decision under appeal.

40. The question arises thus whether or not this submission constitutes an amendment of the appellant's case which might be admitted at the board's discretion only (Article 12(4) RPBA), or even whether the board would be barred from dealing with this issue, because it has been finally decided by the first decision in T 2172/15.

41. The appellant submitted this argument against claim 11 of the main request originally in their notice of

opposition (page 12, sixth paragraph to page 13, first paragraph), albeit under inventive step.

41.1 The opposition division in their first decision under appeal followed the arguments of the respondent on this issue (decision of the opposition division dated 13 October 2015, page 3, second paragraph to page 4, first paragraph).

41.2 The appellant did not object to this part of the decision in their statement of grounds of appeal filed in appeal T 2172/15, or during the second opposition proceedings before the opposition division.

41.3 In T 2172/15, the board set aside the opposition division's decision on sufficiency, considering that the patent did not provide the skilled person, taking common general knowledge into account, with all the information necessary for carrying out the claimed invention over the entire breadth of claim 1 of the main request without undue burden (Article 100(b) EPC). The decision taken on the first appeal thus acquired a *res iudicata* effect between the parties, with respect to the issue of sufficiency of the subject-matter of these claims.

41.4 If a Board of Appeal has issued a decision rejecting certain claimed subject-matter as not being allowable and has remitted the case for further prosecution in accordance with an auxiliary request, submissions of new facts, by introducing for instance new documents or new line of attacks against the same claim wording, are excluded (see also T 449/15, Reasons 2.5; T 1063/92, Reasons 2.5; T 153/93, Reasons 3).

41.5 Indeed the opposition division, in its second decision, correctly indicated that no other reasons for insufficiency had been identified in T 2172/15 (Reasons, 5.5 of the appealed decision) and accordingly did not address this argument.

41.6 The *res iudicata* effect would no longer apply if after remittal for continuation of the proceedings, the claims had been amended, as it was the case for claim 1 of the present auxiliary request 1, thus changing the factual basis of the board's decision. This was however not the case for present claim 10, since, as indicated above (point 31), the identical claim 11 of the main request is on file since the first opposition proceedings and has not been amended. Despite this fact, no objection as to sufficiency has been raised against claim 11 of the main request during the first opposition proceedings, nor during the second opposition proceedings before the opposition division.

41.7 For the sake of completeness, in the present case, even if there were doubts as to whether the first board of appeal decision became final also with regard to the question of sufficiency of the subject-matter of claim 11 of the main request, such an attack would not be admissible for procedural reasons. In view of the fact that the wording of claim 11 of the main request was identical during the whole proceedings, this objection of the appellant under Article 83 EPC against the method of claim 11 of the main request or present claim 10 could and even should have been raised earlier, i.e. during the first appeal proceedings, before decision T 2172/15 was taken (and acquired effect of *res iudicata*). This line of argument will therefore not be considered in these appeal proceedings (Article 12(4) RPBA).

Novelty

42. The appellant submitted that the methods of claims 1 and 11 of the main request lacked novelty over the disclosure of either documents D4 or D5. The same objection is relevant for claims 1 and 10 of auxiliary request 1 too. Since it is uncontested that documents D4 and D5 share a "*similar content*", the following assessment under novelty is limited to document D4, in particular Figures 13 and 27 and passages of document D4 in relation thereto, but applies likewise to the corresponding Figures and passages of document D5.

43. Document D4 concerns a method for sequencing nucleic acids based on so called sequencing-by-synthesis (SBS) reactions. Although fluorescence-labelled nucleotides with reversible terminators are used for SBS, read length limitations - due to diminishing numbers of available templates following each SBS cycle - are a known drawback of this method. As potential solution, document D4 proposes the use of "*template "walking"*" (abstract, page 4, line 6 to page 5, line 15).

43.1 Document D4 discloses that template "walking" in essence describes "*the removal of the sequenced strand and reattaching of the original primer to allow the extension, or walking, of the template with a combination of natural and modified nucleotides to the end of the first round sequence so that SBS can be carried out from that point. Since the original sequenced strand is stripped away, including those terminated with ddNTPs, all the templates become available for "walking". Given that "walking" is carried out with either natural or 3'-modifed nucleotides, the subsequent round of SBS is performed*

on nascent DNA strands for maximum read length" (page 78, line 34 to page 79, line 11).

43.2 Figure 13 of document D4 shows a "*Template "Walking" Method 1*", while Figure 27 of document D4 shows a "*Template "Walking" Method 2 for SBS with C-F-NRTs*" (cleavable fluorescent nucleotide reversible terminators: page 4, lines 20 and 21, page 15, last line, page 17, line 23 and page 81, lines 10 to 23). While the template walking method 1 of Figure 13 uses nucleotides with reversible terminators, the template walking method 2 in Figure 27 uses two sets of natural nucleotides wherein the first set does not contain dTTP and the second set does not contain dCTP (see also page 80, last paragraph to page 81, line 23 and page 89, lines 7 to 16). Due to the presence/absence of reversible terminators during the dark extension step, Figure 13 is relevant for the method of claim 10, while Figure 27 is relevant for the method of claim 1.

43.3 It is uncontested that Figure 27 of document D4 discloses a cycling between two SBS and one template walking on different nascent strands, i.e. a cycling of two read extensions followed by one "dark" extension as defined in steps (a) and (b) of claim 1. It is further uncontested that for any disclosed nascent strand, sequence information is obtained from one region in which a single nucleotide assignment is not made and then one region where at least consecutive positions are assigned with single base resolution.

43.4 The respondent submitted that while Figure 27 disclosed "*one*" region, the functional feature defined by a result to be achieved in claims 1 and 10 mentioned "*regions*", i.e. required a sequence determination from more than one region. While the board agrees with the

respondent that Figure 27 of document D4 discloses explicitly only two read extensions, i.e. two regions wherein all nucleotides are assigned, and one dark extension step with one region of non-assigned nucleotides, Figure 27 is not restricted thereto. Figure 27 mentions at the bottom below the arrow "*Continuous cycle of extension, Identification and cleavage*" which refers to further read and dark extension cycles. Thus Figure 27 when looked at in isolation appears to disclose a method for obtaining sequence information from a target sequence wherein the information contains regions with assigned nucleotides interspersed by regions with non-assigned nucleotides.

44. It is established case law that when assessing the disclosure of a prior art document, no part of this document should be construed in isolation but each part of it should be construed in the context of the contents of the document as a whole (Case Law, I.C. 4.1). The question therefore arises whether or not Figure 27's disclosure changes in the context of document D4's disclosure as a whole.

44.1 As set out above under claim construction (see points 5, 5.1 to 5.3), the functional feature defined by the result to be achieved in claims 1 and 10 is limiting for the subject-matter claimed.

44.2 Document D4 as a whole discloses consistently and exclusively that the rationale behind the combined use of SBS with template "walking" is "*to regenerate the original primer site or to insert two or more primer sites of known sequences into the target DNA so SBS can be carried out at each site sequentially. In general, three steps are involved with this approach: 1) annealing of the first primer, 2) performing SBS, 3)*

denaturing the sequenced section of the template to recover a single-stranded DNA for the second primer annealing. These steps are carried out repeatedly until the target DNA is sequenced in its entirety" (page 69, lines 4 to 15, emphasis added). In other words, each nucleotide in a target sequence is determined, i.e. assigned. This is confirmed by document D4 as a whole which consistently discloses that the "*identity of each of a series of consecutive nucleotide residues in a nucleic acid*" is determined (page 6, lines 4 to 6, emphasis added; also page 8, lines 5 to 7, page 10, lines 1 to 3, or claim 42). Since the skilled person takes the disclosure of document D4 as a whole into account when looking at Figure 27, he/she would have performed sequencing cycles until the complete sequence information of the target nucleic acid was obtained.

45. The facts disclosed in Figure 13 of document D4 are different from those in Figure 27 since regions with non-assigned nucleotides are not disclosed therein. Page 81, lines 3 to 8 states in the context of "*Method 1*" as follows: "*The number of repeated cycles of such incorporation and cleavage will exactly match the actual read length in the first stage of SBS, so that this "filling gap" incorporation stops at the same point where the longest ddNTP primer reaches*" (emphasis added). This means that the "dark" extension step in Figure 13 extends the original sequencing primer exactly up to the position of the nucleotide that has been sequenced in the previous SBS cycle. Since no nucleotides of unknown identity are thus present in the primer walking of Method 1, Figure 13 even looked at in isolation does not anticipate the method of claim 10.

46. Consequently, the sequence information provided by the methods of document D4 is complete, contrary to that

provided by the methods of claims 1 and 10. The same applies to document D5.

47. The methods of claims 1 and 10 are therefore novel and auxiliary request 1 complies with Article 54 EPC.

Inventive step

48. It is uncontested that either document D4 or D5 represents the closest prior art. For the reasons indicated above (point 42) the following assessment of inventive step will be restricted to document D4.

49. The methods of claims 1 and 10 differ from that of document D4 in the functional feature defined by the result to be achieved (point 44.1 above), which has the effect that the identity is not determined for all nucleotides in a target sequence by the claimed methods compared to document D4 (point 46 above). The board agrees thus with the opposition division (decision under appeal, Reasons, point 7.2) that this allows sequence information to be obtained faster.

50. The objective technical problem to be solved resides therefore in the provision of a method for obtaining sequence information faster (decision under appeal, Reasons, point 7.2).

51. The appellant in essence argued that there was no difference between the method of claim 1 and that disclosed in Figure 30 of document D4. The board does not agree. Figure 30 of document D4 shows a "*Template "Walking" Method 5 for SBS with C-F-NRTs*" (page 17, last two lines). This method comprises three normal dNTPs and one nucleotide with a reversible terminator (page 82, lines 5 to 15). Figure 30 discloses in the

second row the use of a dGTP with a reversible terminator for template walking ("dark" read extension) so that nucleotide incorporation stops exactly at that position (i.e. a "*C*") where sequencing ends during the first SBS cycle (row 1, "read" extension). In other words, the target sequence in Figure 30 does not contain regions with non-assigned nucleotides after sequencing.

52. This is in line with the disclosure of document D4 as a whole (point 44.2 above) which reports on methods that provide sequence information for "*each*" nucleotide of a target sequence.

53. Since the methods of document D4 provide a different kind of sequence information compared to the methods of claims 1 and 10 (i.e. a completely determined nucleic acid sequence vs a non-completely determined one), the board is unable to see how the skilled person starting from there and faced with the technical problem defined above would have arrived at the methods of claims 1 and 10 in an obvious manner. In particular, since the appellant's argument under lack of inventive step was in essence an argument under lack of novelty over the disclosure of document D4. For the reasons indicated above, this is however not convincing. Nor does the teaching of document D4 suggest or otherwise point at the use of a dark sequencing reaction in order to obtain sequence information faster but with a sufficient quality on the detriment of the overall quality of the sequence information (complete vs non-complete). The same reasoning applies for a skilled person starting from the teaching of document D5 as closest prior art.

54. The methods of claims 1 and 10 are thus inventive and auxiliary request 1 complies with the requirements of Article 56 EPC.

Reimbursement of the appeal fee

55. Although the appellant has requested a reimbursement of the appeal fee, reasons why this was considered justified have not been submitted.

56. A reason for reimbursing the appeal fee is not apparent to the board either. Particular reference is made to Rule 103(1)(a) EPC, according to which the appeal fee is reimbursed if, in case the board deems the appeal allowable, such is equitable by reason of a substantial procedural violation.

57. Since the appellant has not argued that a substantial procedural violation occurred in the present proceedings and the board is not aware of any either, the appellant's request for reimbursement is rejected.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent in amended form, on the basis of the claims 1 to 11 of auxiliary request 1, filed with the reply to the statement of grounds of appeal, and a description and drawings to be adapted, if needed.

3. The appellant's request for reimbursement of the appeal fee is rejected.

The Registrar: The Chair:

C. Rodríguez Rodríguez T. Sommerfeld

Decision electronically authenticated