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Datasheet for the decision of 1 December 2020

Case Number: T 0127/14 - 3.3.08

Application Number: 96912800.8

Publication Number: 0871646

IPC: C07H21/04, C12P19/34, C12Q1/68

Language of the proceedings: EN

Title of invention:
DNA SEQUENCING BY PARALLEL OLIGONUCLEOTIDE EXTENSIONS

Patent Proprietor:
Solexa, Inc.

Opponent:
Life Technologies Corporation

Headword:
DNA SEQUENCING BY OLIGONUCLEOTIDE EXTENSION/SOLEXA

Relevant legal provisions:
EPC Art. 123(2), 54, 56, 83
RPBA Art. 12(4)

Keyword:
Main request - Novelty (no)
First and second auxiliary requests - Admission into the appeal proceedings (no)
Third auxiliary request - Novelty (no)
Fourth auxiliary request - meets all requirements of the EPC (yes)

Decisions cited:
T 0230/01

Catchword:

Europäisches Patentamt
European Patent Office
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Beschwerdekammern

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Case Number: T 0127/14 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 1 December 2020

Appellant: (Opponent)	Life Technologies Corporation 5791 Van Allen Way Carlsbad, CA 92008 (US)
Representative:	Roques, S.E. J A Kemp LLP 14 South Square Gray's Inn London WC1R 5JJ (GB)
Respondent: (Patent Proprietor)	White, N.L. Solexa, Inc. 25861 Industrial Blvd. Hayward, CA 94545 (US)
Representative:	Boult Wade Tennant LLP Salisbury Square House 8 Salisbury Square London EC4Y 8AP (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 18 November 2013 concerning maintenance of the European Patent No. 0871646 in amended form.**

Composition of the Board:

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

Summary of Facts and Submissions

I. European patent No. 0 871 646 is based on European patent application No. 96912800.8 (published as International patent application WO 1996/033205; hereinafter "the patent application"), and was opposed on the grounds of Articles 100(a), (b) and (c) EPC. The opposition division maintained the patent in amended form. It was held that the main request contravened Article 123(2) EPC, the first auxiliary request contravened Article 83 EPC, the second auxiliary request lacked novelty, and the third auxiliary request complied with the requirements of the EPC.

II. The patent proprietor and the opponent (appellant I and II respectively) lodged appeals against the decision of the opposition division.

III. With their statement of grounds of appeal, appellant I submitted a main request and a first to fourth auxiliary requests.

IV. Both appellants replied to each other's appeals. Appellant I submitted a fifth and sixth auxiliary requests.

V. The parties were summoned to oral proceedings.

In reply thereto, both appellants withdrew their previous requests for oral proceedings.

The oral proceedings were cancelled.

VI. Claims 1 and 11 of the **main request** read as follows:

"1. A method for identifying a sequence of nucleotides in a polynucleotide, the method comprising the steps of:
(a) extending an initializing oligonucleotide along the polynucleotide by ligating an oligonucleotide probe thereto to form an extended duplex which is not capable of ligating to a further oligonucleotide probe;
(b) identifying one or more nucleotides of the polynucleotide;
(c) regenerating an extendable terminus on said extended duplex; and
(d) repeating steps (a)-(c) until the sequence of nucleotides is determined.

11. A method for determining the nucleotide sequence of a polynucleotide, the method comprising the steps of:
(a) providing a template comprising the polynucleotide;
(b) providing an initializing oligonucleotide which forms a duplex with the template adjacent to the polynucleotide;
(c) annealing an oligonucleotide probe to the template adjacent to the initializing oligonucleotide;
(d) ligating the oligonucleotide probe to the initializing oligonucleotide to form an extended duplex which is not capable of ligating to a further oligonucleotide probe;
(e) identifying one or more nucleotides of the polynucleotide by a label on the ligated oligonucleotide probe;
(f) regenerating an extendable terminus on said

oligonucleotide probe; and
(g) repeating steps (c) through (f) until the nucleotide sequence of the polynucleotide is determined."

Dependent claims 2 to 10 and 12 to 15 define specific embodiments of the methods of claims 1 and 11 respectively.

VII. Claims 1 and 11 of the **third auxiliary request** differ from claims 1 and 11 of the main request in that the method of claim 1 step (a) and of claim 11 step (c) require the oligonucleotide probe to have a blocking moiety and in that the method of claim 1 step (b) requires identification of one or more nucleotides of the polynucleotide by a label on the ligated oligonucleotide probe.

VIII. Claims 1 and 11 of the **fourth auxiliary request** are identical to claims 1 and 11 of the third auxiliary request except that the method of claim 1 step (b) requires identification of one or more nucleotides of the polynucleotide by a fluorescent label on the ligated oligonucleotide probe, and in that the method of claim 11 step (e) requires identification of one or more nucleotides of the polynucleotide by a fluorescent label on the ligated oligonucleotide probe.

IX. The following documents are cited in this decision:

D1: WO95/04169 (publication date 9 February 1995)

D2: US 5403708 (publication date 4 April 1995);

D3: WO93/05183 (publication date 18 March 1993).

X. The submissions made by **appellant I**, insofar as relevant to the present decision, may be summarised as follows:

Main request
Article 123(2) EPC

Claims 1 and 11 did not comprise added subject-matter for the reasons set out in the decision under appeal in item 2.2.1.1.

Novelty (Article 54 EPC)

Document D1 described a reagent and a method of using it for sequencing a polynucleotide using mass spectrometry. The reagent was a bivalent linker associated with a nucleotide on one side and a reporter group on the other side of the linker such that the nucleotide at each position within the reagent was labelled "with a unique tag" (see page 5, lines 23-24; Fig.4 and 5). The reagent comprising an oligonucleotide sequence (analyte) and a detectable tag portion was generated by repetitive addition of both nucleotides and reporter groups. Each reporter group in the tag portion identified a nucleotide and its position in the oligonucleotide portion (analyte) of the reagent (see Fig.1 and 5). The nucleic acid sequence determination by progressive ligation of example 1 was the only embodiment potentially relevant for novelty (see Figure 4 and page 14, lines 31 to page 16, line 35). The first step of the method required capture of a target polynucleotide by an immobilised initializing oligonucleotide (see Figure 4 and page 15, line 23). The oligonucleotide portion of the reagent hybridising to the target polynucleotide at a position adjacent to the initializing oligonucleotide along the sequence of

the target polynucleotide (see page 15, lines 34-36 and Figure 4) was then ligated to the initializing oligonucleotide. The subsequent cleavage of the tag moiety and "analysis of the cleaved tag" by mass spectrometry allowed the sequence of nucleotide of the oligonucleotide of the ligated reagent to be determined, revealing thereby the nucleotide sequence of the hybridised section of the target polynucleotide (see page 16, lines 7-9).

Document D1 was however non-enabling, because the technical detail disclosed in document D1 necessary to practice the invention was insufficient.

Although document D1 disclosed a method where steps c) to f) were repeated to determine the nucleotide sequence of a longer target polynucleotide, it remained silent how this repetition had to be achieved. The embodiment on pages 14 to 16 failed to disclose how the cleavage of the tag was carried out and how the extended duplex was returned to conditions enabling another ligation, in particular how the extended duplex is transferred from a dry state, required for the tags' cleavage from the reagent, back into solution for the next ligation step to occur (see page 12, lines 21-23 and page 45, lines 13-20).

Document D1 did not describe furthermore 1) how to determine the sequence of one or more nucleotides of a target polynucleotide based on the sequence of the oligonucleotide used in the reagent; 2) how to prepare a library of labelled oligonucleotide reagents for use in the sequencing method, and 3) how to perform multiple repeated sequencing steps.

The progressive ligation method of document D1 established only that the complete tag moiety was "recovered and analysed" as an indication of the sequence of a first part of a target nucleic acid (see page 16, lines 9-12). The analysis of the tag moiety could however lead to ambiguities if it was fragmented (see page 14, paragraph 1). There was no indication of how a complete tag moiety allowed to determine the identity of the nucleotide at each position of the oligonucleotide portion of the reagent and of how the one or more nucleotides of the target polynucleotide was/were determined.

There was no disclosure how to determine which reporter was present at which specific position in the complete tag moiety to determine the identity of one or more nucleotides of a target polynucleotide. Although example 19 described the detection of a sequence of five thymine residues and a separate sequence of ten adenosine residues using an attached tag (see page 46, lines 12-33), the mass spectrometric analysis of the two tags associated with a specific oligonucleotide would not allow the skilled person to determine the nucleotide sequence of a polynucleotide, as required by claims 1 and 11.

There was no hint in document D1 that the tag synthesis technique described on page 7 or Figure 3 had to be used in the progressive ligation method described on pages 15-16 and Figure 4 (see decision T 305/87 of 1 September 1989). Even if both passages were nevertheless combined the coupling efficiency would cause each hexamer to be linked to a nested set of seven partial synthesis tags each containing a hexameric nucleotide sequence labelled with 0, 1, 2, 3, 4, 5 or 6 reporter groups (see page 7, paragraph 2)

which would prevent the skilled person from deconvolving the partial synthesis products to ascertain the polynucleotide's sequence.

Last, document D1 did not establish how to prepare a library of labelled oligonucleotide reagents for use in a progressive ligation sequencing method. If the coupling efficiency for both the nucleotide and the corresponding reporter to a bivalent linker occurred at 100%, then a library of 4096 hexamers would be enough. If the coupling efficiencies occurred at 100% for the nucleotide and occurred only at 80% for the label, then a library of 28672 labelled hexanucleotides would be needed, as each specific hexanucleotide was linked with a set of tags consisting of up to 6 reporter groups. Hence, the skilled person had to use a large volume comprising a complex mixture of labelled hexamers with only a hope to determine the target polynucleotide's sequence.

Third auxiliary request
Article 123(2) EPC

Claims 1 and 11 did not comprise added subject-matter for the reasons set out in the decision under appeal in item 4.2.1.

Novelty (Article 54 EPC)

The third auxiliary request was not anticipated by the content of document D1 for the same reasons as outlined for the main request.

Fourth auxiliary request
Article 123(2) EPC

Claims 1 and 11 did not comprise added subject-matter for the reasons set out in the decision under appeal in item 5.1.2.

Novelty (Article 54 EPC)

The fourth auxiliary request was not anticipated by the content of document D1, for the same reasons as outlined for the main request.

Inventive step (Article 56 EPC)

The subject-matter of claims 1 and 11 involved an inventive step for the reasons set out in the decision under appeal in item 5.2.

The patent and document D3 related to methods that did not require high resolution electrophoretic separations of DNA fragments (see patent application on page 3, document D3, page 1, lines 6-9). Document D3 represented therefore a suitable starting point.

The method of claim 1 differed from the method of document D3 in that a ligase instead of a polymerase was used to extend the initializing oligonucleotide (see page 3, lines 15-19 and page 9, lines 23-25). The identity of the attached nucleotide was identified by a reporter that could be a fluorescent label (see page 7, line 6). The use of a ligase rather than a polymerase in a sequencing method allowed longer target polynucleotides to be sequenced and prevented problems associated with the use of polymerases e.g. fidelity, processivity and polymerization rate (see page 2, lines 43-56).

Starting from the closest prior art document D3, the technical problem to be solved by the patent was therefore to provide an improved sequencing method which can be used to accurately sequence longer target polynucleotides.

Document D2 disclosed a nucleic acid sequencing method using a ligation reaction to extend a primed template (see column 2, lines 23-61). After a single round of ligation, the identity of the extension oligonucleotide was determined by separating the ligation products using gel electrophoresis (column 2, lines 56-61; column 4, lines 1-6; column 8, lines 5-9) to identify one or more nucleotides of the nucleic acid. It did not contemplate any mechanism for identifying one or more nucleotides of the nucleic acid without using gel electrophoresis.

Thus, starting from the method described in document D3 (see page 1, lines 6-9; page 2, lines 27-29), a skilled person seeking to provide an improved sequencing method, would not have turned to the method of document D2.

Without prior knowledge of the invention claimed the skilled person faced with the technical problem identified above would not have modified the iterative sequencing method of document D3 to include the ligation reaction used in the method of D2, as the gel separation step was mandatory in document D2 whereas it was excluded in document D3.

If the teaching of document D3 were combined with the method of document D1, the skilled person would have used the bivalent reagent and mass tag label disclosed

in document D1 and would definitely not have arrived at a method of claim 1 using a fluorescent label.

XI. The submissions made by **appellant II**, insofar as relevant to the present decision, may be summarised as follows:

Main request
Amendments (Article 123(2) EPC)

The subject-matter of claims 1 and 11 differed from the claims of the patent application in that they specified that the extended duplex was not capable of ligating to a further oligonucleotide probe, and included an additional step of regenerating an extendable terminus on the oligonucleotide probe. These features were only mentioned in the context of a method where a blocking moiety was present and/or which comprised the step of removing the chain-terminating moiety and extending the oligonucleotide probe with a nucleic acid polymerase in the presence of one or more labelled chain-terminating nucleotide triphosphates.

There was no disclosure on page 11 at lines 15 to 17 of the patent application that the prevention of multiple extensions occurred when an extended duplex itself was incapable of ligating to a further oligonucleotide probe. The method of the patent application referred to an oligonucleotide probe that had a blocking moiety preventing multiple probe ligations on the same template in a single extension cycle, which was however not mentioned in claims 1 and 11 (see page 3 lines 30-33,page 7 line 25, page 12, line 5 and page 15, line 2). The patent application failed to disclose a method defined by the specific steps of claims 1 and 11, comprising the function of being incapable of ligating

to a further oligonucleotide probe in combination with the general step of regenerating an extendable terminus on the extended duplex in the absence of the blocking moiety. Thus, the subject-matter of claims 1 and 11 contravened Article 123(2) EPC.

Novelty (Article 54 EPC)

Document D1 described a method of nucleic acid sequencing by sequential ligation (see section beginning on page 14 at line 31 and page 15 lines 1 to 10). The method comprised the steps (a) to (d) of claim 1: hybridising the nucleic acid sequence to be determined or target nucleic acid with an immobilised oligonucleotide (an initialising oligonucleotide), hybridising an oligonucleotide adjacent to the immobilised oligonucleotide (the oligonucleotide probe), and ligating the adjacent oligonucleotide. The oligonucleotide comprised a tag moiety which was analysed to provide an indication of the sequence it hybridized to as set out in step (f) (see page 15, line 12 and page 16, lines 5 to 10). The removal of the linker according to page 16, lines 10 to 14, regenerated an extendable terminus on the extended duplex for another ligation step, which meant that said duplex was not capable of being ligated to any further oligonucleotide probe prior to removal of said linker. The identification of one or more nucleotides of the polynucleotide sequence and the repetition step (d) of claim 1 were described on page 15, lines 12 to 14 and page 16 line 19 of document D1. Hence, the subject-matter of claim 1 was not novel.

Document D1 disclosed all the individual steps of the method of claim 11 as well (see page 14, line 31 to page 16, line 25; especially page 15, lines 1-2).

The "primer" oligonucleotide was chosen to be part of the vector sequence comprising the polynucleotide of interest, wherein the initialising oligonucleotide hybridises to the vector sequence adjacent to the polynucleotide sequence of interest, as claimed in step (b) of claim 11 (see page 15, line 20 and lines 26 to 27). The steps (c), (d) and (e) of claim 11, describing the identification of a nucleotide of the polynucleotide by a tag on the ligated oligonucleotide probe, were disclosed on page 15, lines 3 to 9 and page 16, lines 9 to 10. The regeneration of an extendable terminus and the repetition of method steps (c) to (f) were disclosed on page 16, lines 10 to 14, and line 19. Thus, document D1 disclosed the method steps (f) and (g) of claim 11 too.

Since the extended duplex, resulting from the ligation of an initialising oligonucleotide with an adjacent template oligonucleotide probe, composed of an oligonucleotide moiety, a bivalent linker and a tag moiety, would expose an hydroxyl group or phosphate group only after the linker associated with the tag moiety was removed, said tag moiety linked to the oligonucleotide probe preventing multiple ligations was a chain-terminating moiety at a terminus distal to the initialising oligonucleotide. Thus, document D1 anticipated the subject matter of claims 2 and 12 as well.

Third auxiliary request
Article 123(2) EPC

Claims 1 and 11 added subject-matter beyond the content of the patent application.

Claims 1 and 11 of the third auxiliary request differ from claims 1 and 11 of the main request in that the method of claim 1 step (a) and of claim 11 step (c) require the oligonucleotide probe to have a blocking moiety and in that the method of claim 1 step (b) requires identification of one or more nucleotides of the polynucleotide by a label on the ligated oligonucleotide probe.

Amended claims 1 and 11 infringed Article 123 (2) EPC because the blocking moiety was not at a terminal position of an oligonucleotide and was not specified to first prevent multiple oligonucleotide extensions during a single extension cycle to occur and to be capable, when removed, of regenerating an extendable terminus on the extended duplex (see page 3, line 31, of the patent application).

Since the scope of claim 1 was broader than that of claim 2, which specified that the blocking moiety was a chain terminating moiety at a terminus distal to said initialising oligonucleotide, claim 1 embraced oligonucleotide probes with a blocking moiety at any position. For the above reasons, the third auxiliary request contravened Article 123(2) EPC.

Novelty (Article 54 EPC)

Appellant II repeated the arguments already put forward in the context of the main request.

Fourth auxiliary request
Article 123(2) EPC

The objection under Article 123(2) EPC raised against the third auxiliary request was repeated.

Inventive step (Article 56 EPC)

Document D3 disclosed a method with repetitive cycles in which an initialising probe was extended and contacted with a medium containing a modified nucleotide and an enzyme to attach the modified nucleotide to the 3'-hydroxyl terminus of the primer attached to each template. The modified nucleotides comprised a blocking group and a reporter group which may be a fluorescent dye (see page 6, lines 29 to 30). The blocking group prevented the addition of further nucleotides to the primer (see page 3,lines 18 to 19). The blocking group was removed to regenerate an extendable terminus to allow the method steps to be repeated until the DNA or RNA was sequenced (see page 3, lines 22 to 26, page 11, line 27). The blocking group could be removed by acid/base or light treatment. Thus, it provided an iterative process comprising the steps of extending an oligonucleotide, incorporating a blocking moiety to prevent further extension, an analysis and identification step of the extended sequence, a regeneration of an extendable terminus, and a repetition of these steps.

The difference between the method of document D3 and the method of claim 1 was the use of a probe having more than one nucleotide instead of using an extension reaction adding single nucleotides.

The use of a ligase instead of a polymerase allowed the sequencing of long target polynucleotides and of sequences more distant from the initialising target to be identified. The nucleotides of the probe served as spacers to ensure that the next ligation will take place at a predetermined site or number of bases along

the template (see patent application on page 8, first paragraph).

Starting from the teaching of document D3 and based on the technical effect ascribed to the difference identified above, the technical problem to be solved was to provide an alternative method allowing downstream sequences to be queried.

The solution to this problem was to use a ligation probe instead of the single nucleotide extension reaction as described in document D3.

The use of oligonucleotide probes as spacers to identify sequences distant from an initialising probe was well known in the art. The use of ligation reactions to distinguish between matched and mismatched sequences was equally well known.
Thus, the use of oligonucleotide probes in a ligation reaction instead of a polymerase extension reaction to effectively walk along a sequence at intervals of N nucleotides in order to determine sequences distant from the initial oligonucleotide was a matter of routine and an obvious choice for the skilled person (see document D2, col.4, line 32 et seq.; Figure 2).

The same iterative process using oligonucleotide probes ligated one by one on a polynucleotide template was described in document D1 (see beginning of page 15).

Thus, the method of claim 1 lacked an inventive step over document D3 in combination with either document D2 or D1.

XII. Appellant I requests that the decision under appeal be set aside and that the patent be maintained on the

basis of the main request or one of the first to sixth auxiliary requests.

XIII. Appellant II requests that the decision under appeal be set aside and that the patent be revoked. They request the first, second, fifth and sixth auxiliary requests not to be admitted into the appeal proceedings.

Reasons for the Decision

Main request

The main request is identical to the first auxiliary request underlying the decision under appeal.

Article 123(2) EPC

1. Claims 1 and 11 differ from claims 1 and 13 of the patent application in that step (a) was amended to read: "extending ... to form an extended duplex which is not capable of ligating to a further oligonucleotide probe", and step (c) was amended to read: "regenerating an extendable terminus on said extended duplex".

1.1 Appellant II argued that these two additional features were only mentioned in the context of a method where a blocking moiety was present and/or which comprised the step of removing the chain-terminating moiety and extending the oligonucleotide probe with a nucleic acid polymerase in the presence of one or more labelled chain-terminating nucleotide triphosphates. There was no suggestion on page 11, lines 15 to 17, of the patent application that the prevention of multiple extensions should specifically be conducted by forming an extended duplex which itself is not capable of ligating to a further oligonucleotide probe.

1.2 The board notes that claims 1 and 13 of the patent application relate to methods comprising repeated steps of ligating and identifying one or more nucleotides of the oligonucleotide probes. On page 11, lines 15 to 17, it is explicitly stated that "since the ligation of multiple probes to the same extended duplex in the same step would usually introduce identification problems it is useful to prevent multiple extensions and to regenerate extendable termini." In other words, the extended duplex should not be capable of ligating to a further oligonucleotide. There is no reason why the generic statement on page 11 should be limited by statements in other parts of the description, for example by the features of claims 4 or 14 of the patent application requiring the incorporation of a chain-terminating moiety and an identification by removing the chain-terminating moiety and extending the probe in the presence of one or more labelled chain terminating nucleoside triphosphate.

1.2.1 According to page 11, lines 15 to 17 of the patent application, multiple extensions per cycle are prevented when either the initialising oligonucleotide or the extended duplex resulting from a preceding ligation step is extended by an oligonucleotide probe which is not capable of ligating to a further oligonucleotide probe. Whether the next oligonucleotide probe to be ligated in an extended duplex or the resulting extended duplex after the ligation step is prevented from ligating to a further oligonucleotide probe is irrelevant. In both cases the blocking moiety on the oligonucleotide probe prevents multiple extensions.

Thus, the methods of claims 1 and 11 comply with Article 123(2) EPC.

Novelty (Article 54 EPC)

2. Document D1 discloses a method of nucleic acid sequence determination by progressive ligation of oligonucleotide probes comprising steps (c), (d), (e) and (f) which, after regeneration of an extendable terminus on an extended duplex, may be repeated (see page 16, lines 15 to 25). The repeated method steps involve hybridisation of a reagent from a library, ligation of the reagent, recovery and analysis of a tag chain linked to and cleaved from the reagent, thus generating a 3' hydroxyl or 5' phosphate group for a further ligation. The process can be repeated until the whole DNA sequence is read or the yields in the reaction become too low.

2.1 Appellant I asserted that document D1 was non-enabling because the nucleotide sequence of the reagent could not be deconvolved, libraries of reagents could not be obtained and methods steps (c) to (f) could not be repeated when an extendable terminus on the extended duplex was regenerated. It was equally highlighted that the release of a tag from its analyte moiety by photocleavage occurred in a dry state (see page 12 lines 21-23) while the ligation was performed in solution (see page 45, lines 13-20), but the skilled person was unable to transfer an extended duplex from a dry condition back into a solution whilst retaining the duplex in a viable form for the next round of ligation. Thus, the skilled person was incapable of performing the teaching of document D1 due to a scarcity of technical detail disclosed in document D1.

2.2 In the board's assessment, document D1 describes at least one way how tags can be synthesized to allow the deduction of the nucleotide sequence of the corresponding oligonucleotide sequence and how labelled oligonucleotides can be generated (see page 7, last paragraph and Fig 3). Furthermore, document D1 stresses that the deduction of the oligonucleotide sequence from the tags' composition is facilitated when the coupling of the reporter monomer of the tag on the bivalent linker is incomplete, e.g. 80% (see page 6, last paragraph; Fig 1 and 3). Each oligonucleotide moiety of the reagent will be coupled via a bivalent linker to a set of various tags having an incremental number of reporter the maximum number of which coincides with the maximum number of nucleotides of the oligonucleotide moiety. Since the pool of partial tags generated during the tag's synthesis, corresponding to one oligonucleotide sequence moiety, generates a unique mass spectrometry profile, the sequence of nucleotide residues and their position can be determined. Document D1 describes the production of a library of labelled oligonucleotides in example 14, and the chemical synthesis of the bivalent reagents used in examples 14 to 19 in examples 1 to 13, while example 1 and Fig. 4 explicitly describe the method steps (c), (d), (e) and (f) of accommodating and ligating a second reagent. It describes finally how the recovered tag moiety is analysed by mass spectrometry to determine the nucleic acid sequence of the ligated oligonucleotide (see e.g. example 19).

2.3 There is thus no evidence supporting appellant I's assertion that the nucleotide sequence of the reagent cannot be deconvolved, that libraries of reagents cannot be obtained and that the methods steps (c) to

(f) cannot be repeated when an extendable terminus on the extended duplex is regenerated.

Thus, there is reason to disregard document D1 as allegedly deficient in its disclosure (see decision T 230/01 of 26 April 2005, Reason 5.2).

2.4 It follows that the method for nucleic acid determination by progressive ligation described in example 1 and illustrated in Figure 4 of document D1 anticipates at least the subject-matter of claims 1, 2, 11 and 12.

Admission of the first and second auxiliary requests into the appeal proceedings

3. The first and second auxiliary requests were first filed with the statement of grounds of appeal. Pursuant to Article 25(2) RPBA 2020 in conjunction with Article 12(4) RPBA 2007, the board has a discretion to hold inadmissible facts, evidence or requests, which could have been presented or were not admitted into the first instance proceedings. The board cannot see any reason - and the appellant did not provide any - why the first and second auxiliary requests could not have been submitted in the first instance proceedings. Their submission now is neither occasioned by issues raised for the first time in the decision under appeal nor by arguments raised by the opponent only at a late stage in the first instance proceedings. Hence, the first and second auxiliary requests filed with the appellant's statement of grounds of appeal cannot be taken into account/admitted into the appeal proceedings.

Third auxiliary request

4. The third auxiliary request is identical to the second auxiliary request underlying the decision under appeal and therefore forms part of the appeal proceedings.

Claims 1 and 11 of the third auxiliary request differ from claims 1 and 11 of the main request in that the method of claim 1 step (a) and of claim 11 step (c) requires the oligonucleotide probe to have a blocking moiety, while claim 1 step (b) requires, in addition, the identification of one or more nucleotides of the polynucleotide by a label on the ligated oligonucleotide probe.

Article 123(2) EPC

5. As far as appellant II repeated, in this context, their arguments put forward against the main request, reference is made to point 1 above.

6. Appellant II submitted further that claims 1 and 11 of the third auxiliary request went beyond the content of the patent application because they specified (i) a blocking moiety, which was not necessarily located at a terminal position so that only a single extension of the extended duplex could occur in a single extension cycle (see page 3, line 31, of the patent application), and (ii) further specified that the oligonucleotide probe to be ligated on the polynucleotide was labelled.

7. The board does not share appellant II's view that the blocking moiety of the oligonucleotide probe of amended claim 1 was disconnected from the effect of preventing ligation of a further oligonucleotide probe to the extended duplex and from the regeneration of an extendable terminus of the extended duplex.

8. An oligonucleotide probe having a blocking moiety is disclosed on page 7, lines 25-26 of the patent application where it is stated: "A wide variety of oligonucleotide probes can be used with the invention. ... the probe should possess a blocking moiety to prevent multiple probe ligations on the same template in a single extension cycle ... ". In the light of this teaching, amended claim 1 step (a) does not extend beyond the content of the patent application.
Whether a blocking moiety is characterized differently or more specifically on page 3, line 31 or elsewhere in the patent application is irrelevant in the present circumstance, as the recited basis remains generic.

Feature (ii) of the amended method of claim 1 identified above is based on page 9, line 12 of the patent application, which reads: "The probes of the invention can be labeled in a variety of ways, including the direct or indirect attachment of fluorescent moieties, colorimetric moieties, and the like." The most general statement is thus that probes of the invention can be labelled in various ways. The fact that the various ways of labelling include more specific ways has no limiting effect on the generality of this statement. The mention of more specific ways is merely illustrative. This amendment does not contravene Article 123(2) EPC.

9. In view of these findings, the board concludes that the subject-matter of claims 1 and 11 does not extend beyond the content of the patent application. Thus, the third auxiliary request meets the requirements of Article 123(2) EPC.

Novelty (Article 54 EPC)

10. Appellant II considered that the tag described in the method of document D1 was clearly a blocking moiety, and also a label. Both Appellant I and II maintained otherwise their arguments presented against the main request.

11. As stated in point 2 above, the method of nucleic acid determination by progressive ligation described in document D1 uses a reagent consisting of an oligonucleotide probe and a tag moiety linked by a bivalent linker. Removal of the linker exposes a hydroxyl or phosphate group at the end of the oligonucleotide chain making it available for ligation with the oligonucleotide chain of a second reagent (see page 16, lines 10 to 17). The tag chain is recovered and analysed as an indication of the sequence of a first part of the target DNA. The tag on an extended duplex obtained from the ligation of an initialising probe - or of a previously regenerated extended duplex - prevents multiple extensions. The tag acts as blocking moiety according to claim 1. The removal of the tag from a ligated extended duplex regenerates an extendable terminus on the oligonucleotide chain of the extended duplex which allows another cycle of extension and ligation to occur.
Thus, the method of document D1 cannot be distinguished from the method of claim 1.

12. For these reasons, the subject-matter of claims 1, 2, 11 and 12 is anticipated by the content of document D1.

Fourth auxiliary request

The fourth auxiliary request is identical to the third auxiliary request as upheld by the decision under

appeal and therefore, naturally, forms part of the appeal proceedings.

Article 123(2) EPC

13. Appellant II argued that amended claims 1 and 11 contravened Article 123(2) EPC, for the same reasons as the claims of the third auxiliary request.

13.1 As set out above, the subject matter of claims 1 and 11 does not extend beyond the content of the application as filed.

Novelty (Article 54 EPC)

No objections were raised under Article 54 EPC against this request, and the board can see no reasons to raise any of its own motion.

Inventive step (Article 56 EPC)

Closest prior art

14. It was common ground between the parties that document D3 represents the closest prior art for the assessment of inventive step.

14.1 Document D3 discloses a method with repetitive cycles in which an initialising probe is extended and contacted with a medium containing a modified nucleotide and an enzyme to attach the modified nucleotide to the 3'-hydroxyl terminus of the primer attached to each template. The modified nucleotides comprise a blocking group and a reporter molecule, which may be a fluorescent dye (see page 6, lines 29 to 30). The blocking group prevents the addition of further nucleotides to the primer (see page 3 at lines

18 to 19). The blocking group is removed to regenerate an extendable terminus allowing the method steps to be repeated until the DNA or RNA is sequenced (see page 3, lines 22 to 26; page 11, line 27). The blocking group can be removed by either acid/base or light treatment. Thus, document D3 provides an iterative process comprising the steps of extending an oligonucleotide by incorporating a single nucleotide comprising a fluorescent label and a blocking moiety to prevent further extension, an analysis and identification step of the extended sequence, a regeneration of an extendable terminus, and a repetition of these steps.

14.2 Appellant I and II agreed that one difference between the method of document D3 and the method of claim 1 was the use of a probe being more than one nucleotide in length instead of using an extension reaction adding single nucleotides.

Appellant I stated furthermore that the method of claim 1 implied the use of a ligase instead of a polymerase for extending the initializing oligonucleotide (see page 9, lines 23-25). The use of a ligase instead of a polymerase allowed the sequencing of long target polynucleotides and of sequences more distant from the initialising target to be identified. The nucleotides of the probe served as spacers to ensure that the next ligation would take place at a predetermined site or number of bases along the template (see patent application on page 8, first paragraph). The use of a ligase rather than a polymerase in a sequencing method prevented also other problems associated with the use of polymerases e.g. fidelity, processivity and polymerization rate (see [0006] of the patent).

Hence, appellant I saw the underlying technical problem as the provision of an improved method of sequencing of long target polynucleotides.

14.3 The board is not convinced by appellant I's arguments that the above identified difference leads to an improved method allowing error-free sequencing of long target polynucleotides. There is neither data substantiating that such an improvement occurs indeed when a ligase is used, nor is it mentioned as one of the advancements looked for in paragraph [0008] of the patent. The technical problems reported in [0006] of the background art of the patent included problems caused by the use of nucleic acid polymerases and its properties, but also by the detection and analysis of DNA sequencing fragments present in very small amount, because the signal of the labels was distributed over many hundred spatially separated bands and, in the case of a single lane detection, because of the unavailability of dyes having suitable spectral properties.

14.4 Although, as argued by appellant II, the method of claim 1 might encompass the use of a polymerase extension as specified in dependent claim 3, this step does not form part of the essential steps of the method of claim 1, which however requires the sequence of steps to be repeated at least once. Thus, the use of an oligonucleotide probe acting as a spacer element only to allow the sequencing of polynucleotide sequences distant from an initialising oligonucleotide probe while omitting the steps (b) to (d) of claim 1, is excluded.

14.5 Starting from the teaching of document D3 and based on the technical effect ascribed to the difference

identified above, the technical problem to be solved can be seen as the provision of an alternative method allowing downstream sequences to be queried.

14.6 This problem is solved by the method of claim 1, which uses a ligation probe instead of single nucleotide extension reactions.

14.7 Although the use of oligonucleotide probes as spacers to identify sequences distant from an initialising probe and the use of ligation reactions to allow discrimination between matched and mismatched sequences were known in the art, document D3 provides no incentive to replace the single nucleotide extension reaction used in the method of document D3 by ligation probes.

14.8 Document D2 discloses a nucleic acid sequencing method using a ligation reaction to extend a primed template (see column 2, lines 23-61). After a single round of ligation, the identity of the extension oligonucleotide is determined by separating the ligation products using gel electrophoresis (see column 2, lines 56-61; column 4, lines 1-6; column 8, lines 5-9) to identify one or more nucleotides of the nucleic acid. Document D2 does not contemplate any mechanism for identifying one or more nucleotides of the nucleic acid without using gel electrophoresis.

14.9 Starting from the method of nucleic acid determination without the use of gel electrophoresis described in document D3 (see page 1, lines 6-9; page 2, lines 27-29), and faced with the technical problem identified above, there is no hint or pointer in document D3 prompting the skilled person to turn to the method of document D2 comprising a step of gel separation (see

column 2, lines 56-61; column 4, lines 1-6; column 8, lines 59), and to use oligonucleotide probes and a ligation reaction instead of a polymerase extension reaction to effectively walk along a sequence at intervals of N nucleotides, in order to determine sequences distant from the initial oligonucleotide (see document D2, col. 4, line 32 et seq.; Figure 2).

If, arguendo, the teaching of document D3, relying on repetitive sequencing were nevertheless combined with the method of document D2, the skilled person would have arrived at a method using gel electrophoresis, explicitly stated to be undesirable in document D3, for identifying at least one or more nucleotides after each oligonucleotide extension. However, one would not have arrived at the method of claim 1 or 11, because the separation by gel electrophoresis renders any further extension by repeating the method steps impossible.

It follows that without prior knowledge of the invention claimed, the skilled person would not have modified the iterative sequencing method of document D3 to include the ligation reaction used in the method of document D2 due to the incompatibility between the repetitive sequencing methodology of document D3 and the gel separation step required by the method of document D2.

14.10 Appellant II argued that the same iterative process using oligonucleotide probes ligated one by one on a polynucleotide template was described in document D1 (see beginning of page 15), and could be alternatively combined with the method of document D3.

14.11 The board concurs with appellant I, though, that even if the teaching of document D3 were combined with the

method of D1, using a reagent which allows sequencing of a polynucleotide using mass spectrometry, the skilled person would have used the bivalent reagent with its mass tag label and would not have arrived at the solution of claim 1 using a fluorescent label.

14.12 Thus, the method of claims 1 and 11, and hence the fourth auxiliary request, involves an inventive step over document D3 in combination with either document D2 or D1.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated