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Datasheet for the decision of 11 February 2025

Case Number: T 0822/23 - 3.3.08

Application Number: 17736737.2

Publication Number: 3478853

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Title of invention:
Improvements in or relating to nucleic acid amplification processes

Patent Proprietor:
Lumiradx UK LTD

Opponent:
James Poole Limited

Headword:
Nucleic acid amplification/LUMIRADX

Relevant legal provisions:
EPC Art. 54, 56

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

Decisions cited:

Catchword:

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Boards of Appeal

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Case Number: T 0822/23 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 11 February 2025

Appellant: (Opponent)	James Poole Limited One Southampton Row London WC1B 5HA (GB)
Representative:	A. Strawson Carpmaels & Ransford LLP One Southampton Row London WC1B 5HA (GB)
Respondent: (Patent Proprietor)	Lumiradx UK LTD 3 More London Riverside London SE1 2AQ (GB)
Representative:	Marks & Clerk LLP 15 Fetter Lane London EC4A 1BW (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 27 February 2023 concerning maintenance of the European Patent No. 3478853 in amended form**

Composition of the Board:

Chair	T. Sommerfeld
Members:	M. Montrone
	R. Winkelhofer

Summary of Facts and Submissions

I. An appeal was lodged by the opponent ("appellant") against the interlocutory decision of an opposition division according to which the European patent No. 3 478 853 could be maintained in amended form on the basis of the claims of (then) auxiliary request 1. This patent is based on European patent application No. 17736737.2 which has been filed as International patent application published as WO 2018/002649.

II. The opposition was based on Article 100(a) in conjunction with Articles 54 and 56 EPC, 100(b) and (c) EPC. The opposition division held, *inter alia*, that claim 19 of the main request comprised added subject-matter, while the subject-matter of auxiliary request 1 was held to comply with the requirements of the EPC.

III. With their statement of grounds of appeal, the appellant raised only objections under lack of novelty and inventive step against the subject-matter of auxiliary request 1, as found allowable by the opposition division.

IV. In reply, the patent proprietor ("respondent") re-submitted, *inter alia*, auxiliary request 1 as their (now) main and only request.

V. Claim 1 of the main request reads:

"1. A method of performing a non-isothermal nucleic acid amplification reaction, the method comprising the steps of:

(a) mixing a target sequence with one or more complementary single stranded primers in conditions which permit a hybridisation event in which the primers hybridise to the target, which hybridisation event, directly or indirectly, leads to the formation of a duplex structure comprising two nicking sites disposed at or near opposite ends of the duplex; and performing an amplification process by;

(b) causing a nick at each of said nicking sites in the strands of the duplex;

(c) using a polymerase to extend the nicked strands so as to form newly synthesised nucleic acid, which extension with the polymerase recreates nicking sites;

(d) repeating steps (b) and (c) as desired so as to cause the production of multiple copies of the newly synthesised nucleic acid;

characterised in that the temperature at which the method is performed is non-isothermal, and subject to a reduction of at least 2°C during the amplification process of steps (b)-(d), and wherein the temperature of the reaction does not return to a predetermined temperature".

The subject-matter of dependent claims 2 to 18 is directed to different embodiments of the method of claim 1.

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

VII. Oral proceedings were held with both parties being represented.

VIII. The following documents are referred to here:

D2: US 2009/0017453 A1

D3: WO 2011/030145

D5: US 2003/0082590 A1

D6a: English translation of WO 2007/028833

D7: US 2005/0112631 A1

D8: Buser J.R. *et al.*, Lab Chip, (2015), Vol. 15, 4423-4432

D9: Van Ness J. *et al.*, PNAS, (2003), Vol. 100(8), 4504-4509

D10: "Molecular Beacon Design" webpage; Wayback machine archive; 27 March 2016; https://web.archive.org/web/20160327223139/http:/www.molecular-beacons.org/MB_SC_design.html)

D11: WO 2013/155056

D15: Merriam Webster, "Definition of *vary*"

D17: Han S.-X. *et al.*, Arch. Immunol. Ther. Exp., (2013), Vol. 61, 139-148

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

Main request

Claim construction - claim 1

The features "*subject to a reduction of at least 2°C during the amplification process of steps (b)-(d), and wherein the temperature of the reaction does not return to a predetermined temperature*" in claim 1 did not relate to a unidirectional temperature reduction only. This was in line with the broadest ordinary claim construction since a net lowering by at least 2°C as specified in claim 1 included up- and downward movements (bidirectional) of the temperature profile as long as the temperature did not return to the starting temperature. Nor did claim 1 require a deliberate act of temperature reduction, since any net reduction by at least 2°C was sufficient. For this reason, claim 1 did also not require that the temperature reduction had to be controlled.

Novelty - claim 1

Documents D2 and D11 anticipated the method as defined in claim 1. Both documents disclosed a so-called NEAR (nicking enzyme amplification reaction) amplification which encompassed steps (a) to (d) of claim 1. Although these NEAR amplifications were indicated to be performed under isothermal (i.e. constant) temperature conditions, the temperature was kept only "*essentially*" or "*substantially*" constant without the need for being precisely maintained at one temperature. Both documents indicated that the temperature of the reaction mixture could "*vary by a few degrees*", or "*by only about 1-5°C (e.g. varying by 1, 2, 3, 4, or 5 degrees)*" (document D2, paragraph [0098] and document D11, page 10, lines 10 to 13, respectively). Further, the term "*vary*" used

in documents D2 and D11 according to its ordinary meaning was, for example, synonymous to a change or to modify (document D15). Since a change of temperature necessarily implied either an up- or downward movement of the temperature, the "*at least 2°C*" temperature reduction as defined in claim 1 was directly and unambiguously disclosed in documents D2 and D11.

Inventive step - claim 1

Documents D2 or D11 represented the closest prior art. The alleged distinguishing features to the method of claim 1 were not associated with a technical effect across the whole scope claimed. Claim 1 itself was silent on any technical effect except that it encompassed any method that resulted in a nucleic acid amplification. Steps (a) to (d) referred solely to nicking and extension reactions without indicating further details.
Claim 1 thus encompassed any starting temperature, any extent of temperature reduction, any primer and enzyme, e.g. nickases and polymerases in any ratio and any target sequence (e.g. double stranded or single stranded) to be amplified. Further, no means for detecting the amplification products were indicated in claim 1.
The patent consistently disclosed that the amplification reaction was temperature sensitive (e.g. paragraph [0100]). This was sensible since the function of a nickase needed for the claimed method was temperature dependent. The importance of the start temperature and, in particular, the use of elevated start temperatures was repeatedly mentioned in the patent (e.g. paragraphs [0109], [0122], [0128] and [0136]). However, claim 1 was silent on temperature

requirements, let alone the use of elevated initiation temperatures.

Also the experimental data in the patent disclosed that the initiation temperature was of high importance. Example 7 and Figure 15 of the patent disclosed that at a start temperature of 64°C no increased amplification occurred. Reasons for this were that the nickase had its optimum temperature at around 50°C. Figure 3 of the patent even disclosed that at 50°C starting temperature no amplification occurred. Based on this data in the patent it was not credible that beneficial effects were achievable across the whole temperature range encompassed by claim 1.

As regards the data supporting a beneficial effect of the claimed method, the patent disclosed an increased amplification reaction using 60°C, 62°C or 63°C as a start temperature. Amplification reactions with higher or lower start temperatures were not disclosed. Since Figure 3 of the patent disclosed that when starting from 50°C no amplification occurred and hardly any when starting from 64°C, it was not credible that a temperature lowering by 2°C would have changed that fact. Looking at the limited experimental data provided in the patent, this data rather showed that elevated start temperatures were responsible for the beneficial effects of the claimed method, but not a temperature reduction.

The patent did not even disclose experimental data that a temperature reduction in steps (b) to (d) of claim 1 were associated with a beneficial effect, because this had not been tested. The experiments in the patent solely disclosed that the temperature reduction started in step (a) and not in steps (b) to (d) as mentioned in claim 1. This was derivable from paragraph [0101] and Figure 2A of the patent which disclosed that the enzymes needed for the amplification (nickase and

polymerase) were already present in the initiation reaction, i.e. in step (a) of claim 1. Nor did the patent disclose data for a temperature reduction of at least 2°C.
Since the experimental evidence derivable from the patent was thus rather limited, it was also for this reason not credible that a beneficial effect was associated with the distinguishing features across the whole breadth claimed.
The technical problem to be solved was thus the provision of an alternative amplification method.

Even if the objective technical problem was held to be an improved amplification method, the skilled person would have reduced the temperature during the amplification reaction, for various reasons.

Firstly, the prior art taught that a temperature reduction was inevitable in certain situations. For example, document D2 mentioned that isothermal amplification reactions were not necessarily held at constant temperatures, but performed within a controlled temperature range. Document D8, for example, reported on a precision device that was used for controlling isothermal reactions within a narrow temperature range (~51°C ± 4°C, page 4424, right column, second paragraph). Figure 2A of document D8 disclosed a temperature profile of this device where during the "hold" phase the temperature gradually decreased by at least 2°C. The skilled person combining the teaching of documents D2 and D8 would have thus inevitably arrived at subject-matter encompassed by claim 1. This likewise happened upon combining the teaching of document D2 with document D5 (paragraph [0132]) or with D7 (paragraph [0091]).

Secondly, the prior art taught that the use of an elevated start temperature was beneficial. Document D2, for example, reported in the context of an isothermal reaction that an increased amplification rate was obtained due to a hot start of the reaction followed by an amplification at lower temperatures (e.g. paragraphs [0119],[0118] and [0152]). Similar benefits using an elevated start temperature were mentioned in documents D6a (page 6, last paragraph) and D3 (page 2, lines 3 to 10). The existence of different optimum temperatures for polymerases and nicking enzymes was, for example, mentioned in document D9 (page 4506, left column, second paragraph). In these circumstances, the skilled person would have inevitably arrived at the subject-matter claimed using elevated start temperatures for the isothermal reaction followed by an amplification at lower temperatures.

Thirdly, the prior art further taught that a temperature change improved the detection of amplified products. Document D10, for example, disclosed that a beacon had an optimum sensitivity below 55°C (page 1, first paragraph and Figure). Likewise, document D2 taught that reaction conditions had to be optimised for detecting beacons (Figure 10) and that detection was best at 57.5°C, compared to 50°C or 45°C. A similar disclosure was derivable from document D17 (page 141, right column). Thus also in this situation, the skilled person would have inevitably arrived at the subject-matter claimed by combining for the isothermal reaction elevated start temperatures and lower temperatures during amplification for optimising a beacon-based detection of the amplified product.

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

Main request

Claim construction - claim 1

The terms "*non-isothermal*" and a temperature reduction "*of at least 2°C*" in the context of claim 1, when ordinarily interpreted by the skilled person, meant that said reduction was a deliberate act of a net temperature lowering over the course of the amplification reaction. This interpretation was supported by the indication in claim 1 that the temperature of the reaction did not return to a predetermined temperature.

Novelty - claim 1

The method of claim 1 differed from the NEAR amplifications of documents D2 and D11 in three features: the amplification reaction was "*non-isothermal*", the temperature was reduced by "*at least 2°C*" and the temperature did not return to a predetermined temperature. These features were not directly and unambiguously derivable from documents D2 and D11, since they consistently disclosed that the amplification reaction was performed under isothermal (i.e. constant temperature) conditions. The term "*vary*" mentioned in this context in paragraph [0098] of document D2 and on page 10 of document D11 meant that the temperature varied around one temperature, i.e. cycled around this temperature in a controlled bi-directional manner.
Document D15 was irrelevant for construing the term "*vary*" in documents D2 and D11, since in the context of these documents the term isothermal implied that the temperature was kept essentially constant by cycling around a preset temperature, i.e. a bidirectional

movement. Furthermore, claim 1 used the term reduction, and not change or variation like documents D2 and D11. A reduction implied a unidirectional lowering of temperature.

Inventive step - claim 1

Document D11 did not represent the closest prior art since it taught that for improving the performance of the NEAR reaction, primers were needed containing 2'-modified nucleotides. D11 did not contain a suggestion that the amplification might be improved by reducing the temperature during amplification. Document D2 represented the closest prior art. The claimed method differed therefrom by a temperature reduction of at least 2°C during the amplification reaction which achieved an improved amplification across the whole breadth of claim 1. The burden of proof for showing otherwise rested upon the appellant, because the patent provided experimental evidence thereof.
Also, Example 7 and Figure 15 of the patent disclosed an improved amplification of the claimed method, although the reaction was performed under conditions that were not ideal for isothermal reactions. The fact that the improvement was only small under these conditions was irrelevant for inventive step, since they lied outside of the standard conditions applied for amplification reactions. Likewise, the data disclosed in Figure 3 of the patent were irrelevant for the issue of whether the distinguishing feature achieved an effect across the whole breadth claimed. This Figure showed an isothermal amplification reaction, i.e. a reaction that was not encompassed by claim 1. When comparing the amplification rate under the same reaction conditions, except for a temperature reduction, the claimed method (e.g. Figure 6 of patent)

consistently achieved an improved amplification vis-a-vis an isothermal reaction (Figures 4 and 5 of patent). Since the temperature reduction was the sole difference between these experiments, this reduction was responsible for the observed beneficial effect.
The patent disclosed in Figure 6 also that a reduction by 2°C was sufficient for achieving an improved amplification.
Step (a) of claim 1 did not mention a nickase and a polymerase. These enzymes were added in step (b) and (c) of claim 1 only. This was supported by paragraphs [0096] and [0097] of the patent which disclosed in combination with Figure 2 that the claimed method underwent two distinct phases, an initiation phase and an exponential amplification phase. The nickase and the polymerase were needed for the later exponential phase only, not for the initiation phase.

The technical problem to be solved resided thus in the provision of an improved method.

The method of claim 1 was not an obvious solution to this problem.

Neither the teaching of document D2 nor that of document D11 suggested that a reduction in temperature by at least 2°C during the amplification reaction resulted in an improved amplification. This was also not suggested by combining the teaching of these documents with either one of documents D3, D5, D6a, or D7 to D10.
Document D3 disclosed a method of temperature cycling, i.e. not a temperature reduction which did not return to a predetermined temperature.
Document D5 disclosed isothermal amplifications wherein the temperature was kept essentially constant. The

mentioned ± 20°C temperature variation in paragraph [0132] was inconsistent with the common meaning of isothermal and rather related to the overall temperature range used for isothermal reactions.
Document D6a did not disclose a temperature reduction as specified by steps (b) to (d) of claim 1, i.e. during the amplification reaction.
Document D7 disclosed a random variation or cycling of the temperature around a desired temperature.
Document D8 disclosed an isothermal reaction that operated at about 51°C ± 4°C, i.e. a cycling around a desired temperature too.
Document D10 was solely concerned with the design of molecular beacons and their adaptation to specific conditions of an amplification reaction but not *vice versa*.

XI. The relevant requests of the parties for this decision are:

(a) The appellant requests that:
- the decision under appeal be set aside and amended such that the patent be revoked.

(b) The respondent requests that:
- the appeal be dismissed.

Reasons for the Decision

Main request

Claim construction - claim 1

1. Claim 1 is directed to a "*method of performing a non-isothermal nucleic acid amplification reaction*" which

comprises process steps (a) to (d) (for the full wording of the claim, see section V above). Further process steps are not excluded from the method of claim 1 due to the use of the "*comprising*" language.

1.1 The term "*non-isothermal*" in claim 1 is further specified in that it relates to "*the temperature at which the method is performed*", which is "*subject to a reduction of at least 2°C during the amplification process of steps (b)-(d), and wherein the temperature of the reaction does not return to a predetermined temperature*".

1.2 Process steps (a) to (d) of claim 1 are in essence directed at an amplification reaction characterised by repeated cycles of primer hybridisation, nicking and polymerase-based extension reactions.

2. It is uncontested that the term "*amplification reaction*" (emphasis added) in the preamble of claim 1 relates to the entire method, while the term "*amplification process*" (emphasis added) in the characterising part of claim 1 relates to process steps (b) to (d).

3. The meaning of the term "*non-isothermal*" in claim 1 is contested between the parties. The board agrees with the opposition division (decision under appeal, point 42) and the respondent that a "*non-isothermal*" amplification which is "*subject to a reduction of at least 2°C*" as indicated in claim 1 according to its ordinary meaning relates to an unidirectional act of a net lowering of the temperature by at least 2°C during the amplification process (steps (b) to (d)). This understanding is also supported by the functional requirement in claim 1 that "*the temperature of the*

reaction does not return to a predetermined temperature".

3.1 The appellant's arguments that this encompassed a bidirectional net lowering of the temperature are not convincing, since this goes against the explicit wording of the claim.

3.2 Further, while a net reduction of at least 2°C as indicated in claim 1 relates indeed to a small change that may lie within the normal variation of laboratory methods, an unidirectional temperature lowering of 2°C is not inevitable. In the absence of the explicit requirement that the temperature is not allowed to return to the start temperature as set out in claim 1, temperature variations occurring normally in laboratory methods may go in both directions, i.e. upward and downward.

3.3 Further, there is indeed no requirement in claim 1 that the reduction by at least 2°C happens in a controlled manner (this is indicated in claim 3 only and, hence, an optional feature of claim 1), as likewise supported by paragraph [0065] of the patent. However, the functional requirement in claim 1 that the temperature is not allowed to return to a predetermined temperature (point 3 above) implies that appropriate conditions have to be selected that exclude such a return, for example, by using an active cooling or a passive heat-loss during the process (paragraphs [0066] and [0077] of the patent). Irrespective of whether the means selected for the temperature reduction are applied in a controlled or uncontrolled manner, claim 1 functionally requires that these means result in a reduction of the temperature by at least 2°C (active or passive), i.e.

an unidirectional net lowering of the temperature by at least 2°C during the amplification process.

4. The appellant's submission that the term "*subject to*" in claim 1 related to open language, implying that the reaction was merely susceptible to a reduction, is not persuasive either. The term "*subject to*" in claim 1 specifies a requirement, here the reduction of the reaction temperature by at least 2°C.

5. Further, the temperature reduction of at least 2°C relates to steps (b) to (d) of claim 1 only. This is also in line with the patent's disclosure (paragraphs [0063] to [0065]).

Novelty - claim 1

6. The appellant submitted that the method of claim 1 lacked novelty over the disclosure of documents D2 and D11.

7. As regards document D2, it is uncontested that this document discloses a nicking enzyme amplification reaction ("NEAR"), and that process steps (a) to (d) of claim 1 are disclosed therein.

8. It is, however, contested between the parties whether or not document D2 discloses a "*non-isothermal*" amplification process which is "*subject to a reduction of at least 2°C*". Document D2 consistently mentions that the amplification was carried out at a "*constant temperature*" (e.g. abstract), i.e. at "*isothermal conditions*". Document D2 states in that context: "*By "constant temperature", "isothermal conditions" or "isothermally" is meant a set of reaction conditions where the temperature of the reaction is kept*

essentially constant during the course of the amplification reaction. An advantage of the amplification method of the present invention is that the temperature does not need to be cycled between an upper temperature and a lower temperature. The nicking and the extension reaction will work at the same temperature or within the same narrow temperature range. However, it is not necessary that the temperature be maintained at precisely one temperature. If the equipment used to maintain an elevated temperature allows the temperature of the reaction mixture to vary by a few degrees this is not detrimental to the amplification reaction, and may still be considered to be an isothermal reaction" (paragraph [0098], emphasis added).

8.1 According to the appellant, the term "*vary by a few degrees*" in this passage of document D2 implies a reduction by at least 2°C, which directly and unambiguously anticipates the temperature reduction as defined in claim 1.

8.2 However, the opposition division (decision under appeal, points 43 and 44) and the respondent were right that the term "*vary*" in paragraph [0098] of document D2 implies a bidirectional change, i.e. an upward and downward temperature cycling around a predetermined temperature, and not a net "*reduction*" (i.e. an unidirectional change) as set out in claim 1. Both terms have thus no equivalent meaning. The definition of "*vary*" in document D15 which, for example, discloses that the terms "*change*" and "*modify*" are synonyms of "*vary*", does not change this conclusion. Terms in a document (here document D2) have to be interpreted in their specific technical context. The skilled person reading document D2 knows from their common general

knowledge that isothermal reactions are performed at an essentially constant temperature. This is also addressed in paragraph [0098] of document D2 ("*temperature be maintained at precisely one temperature*") above. The term "*vary*" used in document D2 in its proper context thus implies that the temperature may cycle by a few degrees Celsius around a preset value which, however, does not anticipate the claimed temperature reduction.

9. Similar considerations apply for document D11. It is uncontested that also document D11 discloses a NEAR amplification method that comprises steps (a) to (d) of claim 1. The amplification is carried out at "*substantially isothermal conditions*" (page 25, lines 24 to 26) which is defined as: "*a single temperature or within a narrow range of temperatures that does not vary significantly. In one embodiment, a reaction carried out under substantially isothermal conditions is carried out at a temperature that varies by only about 1-5° C (e.g., varying by 1, 2, 3, 4, or 5 degrees)*" (document D11, page 10, lines 10 to 13, emphasis added). For the same reasons as set out above for document D2, the appellant's arguments cannot be agreed with that document D11 directly and unambiguously disclosed a temperature reduction of up to 5°C. Instead, a cycling is disclosed around a desired temperature within a variation range of 1°C to 5°C.

10. The appellant further argued that the feature "*the temperature of the reaction does not return to a predetermined temperature*" in claim 1 merely meant that the amplification was not based on thermal cycling. Accordingly, any isothermal amplification reaction (which lacked a thermal cycling too) as, for example,

disclosed in documents D2 and D11, implicitly encompassed this feature.

This argument is not convincing either, since the appellant looks at this feature in claim 1 in isolation but not in the context of claim 1 as a whole. Claim 1 not only requires that the temperature does not return to a predetermined temperature, but further specifies that the temperature during the amplification process has to be reduced at least by 2°C (points 3, 3.2 and 3.3 above). This implies that the start and end temperatures of the amplification process as defined in claim 1 have to be different by at least 2°C, a feature that is not implicit in the isothermal amplification reactions disclosed in documents D2 and D11 for the reasons given above (points 8.2 and 9).

11. The main request thus complies with the requirements of Article 54 EPC.

Inventive step - claim 1

12. The appellant submitted that the claimed method lacked an inventive step starting either from document D2 or D11 as closest prior art alone, or by combining the teaching of documents D2 or D11 with documents D3, D5, D6a, or D7 to D10.

Closest prior art

13. The respondent argued that document D11 was not a suitable starting point for the discussion of inventive step. This question does not pose, though, as the appellant has raised objections for a lack of inventive step based on, *inter alia,* document D11 as the closest prior art; since an inventive step can only be

acknowledged if the claimed subject-matter is not obvious having regard to any prior art, it has to be shown that this is the case over document D11's teaching as well. This is indeed the case (see below).

Technical problem

14. The appellant argued that the method of claim 1 differed from those disclosed in documents D2 and D11 only in "*a reduction of at least 2°C during the amplification process (b)-(d)*", but not in that "*the temperature of the reaction does not return to a predetermined temperature*". This is not convincing for the reasons given above under novelty (points 8.2, 9 and 10). Thus the respondent is right that these two features of claim 1 distinguish the claimed subject-matter from that of documents D2 and D11.

15. The appellant also argued that these distinguishing features were not associated with a technical effect across the whole breadth claimed. Reasons for this assertion were essentially that the patent did not demonstrate evidence for such an effect, because (1) limited conditions had been tested and (2) data disclosed in the patent itself did not render it credible that technical effects were associated with the distinguishing features over the whole breadth of the claim. Furthermore (3), if the patent disclosed an effect at all, this was rather caused by an elevated initiation temperature and not by a reduced temperature during the amplification process.

16. As regards the appellant's first line of arguments (point 15 above), and as correctly held by the opposition division (decision under appeal, Reasons 58 to 64), the working examples in the patent compare the

amplification efficiency of a non-isothermal method of the invention named "STAR" for selective temperature amplification reaction (paragraph [0092] of the patent) with an isothermal NEAR amplification, i.e. a method resembling closely that of documents D2 and D11. It is uncontested that the experimental conditions applied in these working examples (Examples 1 and 2 of the patent) are identical for the STAR and NEAR amplifications, except for a net reduction of the temperature during the amplification process of the STAR method over a range of 4°C to 40°C (Table 1 of the patent).

16.1 Further in agreement with the opposition division (decision under appeal, Reason 82) and contrary to the appellant's assertion, Figure 6 of the patent discloses that a 2°C reduction (reached after the assay did run for 5 minutes) of the STAR method results in a stronger amplification signal when compared to the isothermal NEAR method shown in Figure 5 at the respective time point. The patent thus discloses supportive experimental data for the lower range limit of the temperature reduction mentioned in claim 1.

16.2 Moreover, these beneficial effects shown in the working examples have apparently been obtained by a temperature reduction that occurred during steps (b) to (d) of claim 1, i.e. the amplification process (decision under appeal, Reasons 92 to 94). Step (a) of claim 1 does not require the presence of a nicking enzyme and a polymerase. A nicking enzyme is indirectly mentioned for the first time in step (b) of claim 1 ("*causing a nick*"), while step (c) mentions for the first time a polymerase.

16.3 Contrary to the appellant's arguments, this is in agreement with the description in the patent setting

out the experimental conditions applied. Paragraph [0101] mentions only in step 7) "*seal and initiate preselected temperature profile and data collection*" and paragraph [0102] states in this context that during "*the course of a reaction amplified product was measured every 15 seconds by using the molecular beacon as described above*". This wording implies that the temperature reduction occurred only during the amplification process (steps (b) to (d) of claim 1) and not during the primer hybridisation (step (a)) as further supported by Figures 3 to 11 and the corresponding description in paragraphs [0104] to [0107] of the patent. This does not change, though, by the teaching in paragraphs [0096] and [0097] of the patent providing explanations for Figures 2A and 2B, because there the patent describes in general the claimed method, without mentioning a temperature reduction or steps (a) to (d) of claim 1. Thus no conclusions can be drawn therefrom concerning the actual start of the temperature reduction.

16.4 Lastly, the sole use of 60°C to 64°C in Examples 1 to 7 of the patent as initiation temperature for the claimed STAR method is not too limited for casting doubts as to the obtained technical effects over the whole breadth of claim 1 either. As correctly held by the opposition division, it belongs to the skilled person's common general knowledge that the enzymes of the claimed method, i.e. the nickase and the polymerase, require certain standard conditions to be enzymatically active. The same applies to the hybridisation of primers to their target sequences. The skilled person would therefore select these standard conditions for carrying out the claimed method including an appropriate initiation temperature for avoiding, for example, protein denaturation (decision under appeal, Reasons 85

and 86). Indications are also lacking from the documents on file that the initiation temperature selected in the working examples of the patent is non-standard (except for Example 7 and Figure 15, point 17.2 below), or suitable for the STAR method but not for the NEAR method. This has also not been argued by the appellant. In fact, the compounds (e.g. enzymes or primers) used in both methods are the same.

16.5 The consistent experimental data provided in the patent, in the absence of any evidence to the contrary, renders it credible that the data in the patent support over the whole breadth claimed that the temperature reduction is responsible for the beneficial effects.

17. As regards the appellant's second line of argument (point 15 above), the appellant essentially asserted that Figures 3 and 15 of the patent disclosed evidence that the claimed method did not work across substantially the whole temperature range encompassed by claim 1.

18. This is not convincing either.

18.1 Firstly, in agreement with the respondent, Figure 3 of the patent relates to a method that is not encompassed by the method of claim 1. Thus, no conclusions can be drawn therefrom as regards the issue at hand.

18.2 Secondly, as set out above in point 16.4, the skilled person is aware that the enzymes of the claimed method do not work properly under non-suitable conditions, which include a too high initiation temperature. This issue is also addressed in the patent which reports in the context of Example 7 and Figure 15 that "*A further benefit of STAR technology is the ability to amplify*

outside most common isothermal amplification ranges" (paragraph [0129], emphasis added) and that "*It is apparent from the Figures (sic Figure 15A to C) that an initial temperature of 62 or even 63°C provides good results for STAR reactions, and there is even some amplification using an initial temperature of 64°C although this is clearly sub-optimal*" (paragraph [0132], emphasis added). Thus Example 7 of the patent states itself that the conditions used are not common, i.e. non-standard for isothermal amplifications. This includes the claimed non-isothermal amplification method that, except for a reduced temperature during the amplification process, relies on the same enzymes and chemical reactions (point 16.4 above).

18.3 The appellant's reliance on such non-standard conditions to prove that not substantially all of the claimed embodiments showed the beneficial effects is not convincing, since these conditions would be disregarded by the skilled person.

19. As regards the appellant's third line of argument (point 15 above), the following is relevant.

19.1 As set out above (point 16), the patent discloses in Table 1 a comparison between the claimed non-isothermal amplifications and isothermal amplifications wherein the sole distinction resides in a temperature reduction as specified in claim 1. Examples 1 and 2 and Figures 5 and 6 of the patent disclose that the amplification efficiency of the non-isothermal amplification is improved, compared to an isothermal amplification.

19.2 Further, as also correctly held by the opposition division, the fact that 60°C as start temperature of the non-isothermal reaction and of one of the three

isothermal reactions (Table 1 of the patent) lies outside of the optimum temperature of the nickase is irrelevant for the present case, since this applies for the polymerase too (decision under appeal, Reasons 84 to 86). Further as set out above (point 16.5), in view of the consistent data provided in the patent for an effect being associated with the temperature reduction and the lack of evidence to the contrary, the appellant's argument that an elevated initiation temperature would be responsible for the improved amplification efficiency is not persuasive either.

20. Owing to these considerations, the opposition division was right to conclude that the distinguishing feature of the claimed method is associated with a beneficial effect over substantially the whole breadth of claim 1. Thus, also in agreement with the opposition division, the technical problem to be solved resides in the provision of an improved amplification method (decision under appeal, Reasons 99 and 100).

21. In view of the experimental data provided in the patent, the method of claim 1 solves this technical problem.

Obviousness

22. The appellant submitted that even if the problem resided in the provision of an improved amplification method, the claimed method was obvious over the cited prior art, because the skilled person starting from the method of either of documents D2 or D11 was motivated to reduce the temperature during the amplification process. Reasons for this were:

- (1) that beneficial effects of elevated initiation temperatures were known (documents D2, D3, D6a and D9),
- (2) the known improved detection sensitivity of products generated at reduced amplification temperatures (documents D10 and D17),
- (3) the inevitable arrival at reduced temperatures during the amplification process (documents D5, D7 and D8).

23. This is not persuasive either.

24. It is established case law that when considering whether or not claimed subject-matter constitutes an obvious solution to an objective technical problem, the question to be answered is whether or not the skilled person, in the expectation of solving the problem, would have modified the teaching in the closest prior art document in the light of other teachings in the prior art so as to arrive at the claimed invention. Therefore, the point is not whether the skilled person could have arrived at the invention by modifying the prior art, but rather whether, in expectation of the advantages actually achieved (i.e. in the light of the technical problem addressed), would have done so because of promptings in the prior art (Case Law of the Boards of Appeal of the EPO, 10th edition 2022, I.D.5).

25. Documents D2 and D11 are silent on any suggestion for reducing the temperature during the amplification process for potentially improving the efficiency of the reaction. The appellant submitted in this context that document D2 disclosed a so-called "hot start" (e.g. paragraphs [0011] and [0118]), for example, for denaturing double stranded target molecules before initiating the isothermal amplification.

However, the method of claim 1 is silent on using a "hot start" to denature the template before the amplification starts. Even if the claimed method might encompass such a hot start due to the use of the "comprising" language, document D2 discloses that the isothermal amplification process is performed after the hot start (e.g. paragraph [0119]), i.e. the amplification is performed at a substantially constant temperature. Contrary thereto, the claimed method requires that the temperature reduction occurs during the amplification process as specified by steps (b) to (d) of claim 1. Thus, based on the teaching of documents D2 or D11 alone, the skilled person would not have arrived at the claimed method in an obvious manner.

26. The issue thus arises whether the skilled person would have arrived at the claimed method in an obvious manner when combining the teaching of either document D2 or D11 with one of the other cited prior art documents.

27. As regards the first line of argument (point 22 above), the appellant submitted that also documents D3 (page 2, lines 8 to 13) and D6a (page 6, last paragraph to page 7, first paragraph) disclosed a so-called "hot start".

27.1 Document D3 mentions that a hot start may precede the isothermal amplification (page 2, lines 8 to 10), which is based on a "*temperature oscillation*" (title and abstract), i.e. a temperature cycling. Document D3 describes this reaction as "*a method for carrying out an isothermal nucleic acid amplification reaction at a predetermined temperature, said method comprising changing the temperature of the reaction mixture away from the said predetermined temperature and allowing it*

to return to the predetermined temperature at least once during the amplification reaction" (page 3, lines 8 to 12, emphasis added). Document D3 discloses thus an essentially isothermal reaction wherein at least once during the reaction the temperature is changed, i.e. becomes non-isothermal. The temperature must, however, return to a predetermined temperature. This does not change with the statement on page 3, lines 21 to 22, which reads "*In a particular embodiment, the temperature is allowed to move in a downward direction*", since this solely specifies the direction of the temperature change without indicating that after this change there is no return to the predetermined temperature. Contrary thereto, the claimed method requires that the temperature reduction occurs during the amplification process as specified by steps (b) to (d) of claim 1 without returning to a predetermined temperature.

27.2 Also document D6a discloses that the isothermal amplification reaction is performed after the hot start, i.e. the amplification is performed at a substantially constant temperature (e.g. page 8, line 23 to page 9, line 2). Thus, the same reasoning applies as set out above for document D2 (point 25).

27.3 Document D9 discloses isothermal reactions and that the nickase and polymerase used in these reactions have different optimum temperatures (abstract and page 4506, left column, second paragraph). As regards the yield of the reaction and how to optimise it, document D9 states "*that the reaction is completely dependent on the presence of both enzymes, the template, and the primer oligonucleotide (data not shown), but the yield is a complex function of the amounts of both enzymes. ... In addition, it is clear from the data (...) that we can*

modulate the yield of partial products by changing the ratio of the enzymes. Although we do not know precisely how the enzymes interact, cooperate, or compete with one another, it is clear that there are optimal concentration ranges of both enzymes" (page 4506, right column, first paragraph, emphasis added). In other words, document D9 suggests for an optimised yield of the isothermal amplification that the concentration ratio of the nickase and the polymerase must be optimised. This is different from a temperature reduction during the amplification process as mentioned in the claimed method.

27.4 Owing to these considerations, the appellant's first line of argument is not convincing.

28. As regards the second line of argument (point 22 above), this is not convincing either, for the following reasons.

28.1 The method of claim 1 is silent on the use of any detection means for the amplified product.

28.2 Even if the claimed method encompassed the use of such means, due to the "comprising" language, document D10 is concerned with molecular beacon design (title), not with the design of nucleic acid amplifications, let alone their optimisation. This is evident from various statements in document D10 that read: "*In order to design molecular beacons that function optimally under a given set of assay conditions*"; "*The process of molecular beacon design begins with the selection of the probe sequence*"; and "*After selecting the probe sequence, two complementary arm sequences are added on either side of the probe sequence*" (page 1, first sentences of first to third paragraph respectively).

Thus, the skilled person reading the teaching of documents D2 or D11 and combining it with that of document D10 is taught that the design of a molecular beacon must be adjusted for adapting the beacon to the specific isothermal reaction conditions reported in documents D2 or D11. However, pointers for adjusting document D2 or D11's amplification conditions for adapting them to a specific molecular beacon are lacking from documents D2, D11 and D10.

28.3 The same considerations apply for document D17 which concerns a review article about molecular beacons as diagnostic tool (abstract and title). The generic teaching in document D17 (page 141, right column, second to fourth paragraph) that, *inter alia,* the temperature and the environmental pH are amongst the most important impact factors for the functioning of molecular beacons does not point to a temperature reduction during amplification. Rather, the skilled person would be motivated to design a beacon that fits the amplification conditions of the assay.

29. Lastly, also the appellant's third line of argument (point 22 above) is not convincing, for the following reasons.

29.1 Document D5 teaches isothermal amplification reactions. Paragraph [0132] of this document defines "*isothermal conditions*" as reactions wherein the temperature is "*kept essentially constant (i.e., at the same temperature or within the same narrow temperature range wherein the difference between an upper temperature and a lower temperature is no more than 20° C)*". Although this definition indicates a range of ± 20°C as being isothermal, this passage does not state that a single isothermal reaction varies by ± 20°C. Such an

interpretation would also be inconsistent with the definition of isothermal conditions that keep the temperature "*essentially constant*" or "*within the same narrow temperature range*". The range of ± 20°C rather refers to the overall temperature range used for isothermal amplifications as supported by a further statement in paragraph [0132], reading that "*Exemplary temperatures for isothermal amplification include, but are not limited to, any temperature between 50° C. to 70° C*". Indications that isothermal reactions inevitably result in temperature reductions during amplification are thus missing from document D5.

29.2 Similar considerations apply for the teaching of document D7, which discloses an isothermal amplification reaction that is termed "*RPA*" (recombinase polymerase amplification, paragraph [0013]), i.e. an amplification reaction that uses a recombinase instead of a nickase, as does the claimed method. Document D7 states that the RPA method is advantageous because "*the temperature is not critical and precise control, while preferred, is not absolutely necessary. For example, in a field environment, it is sufficient to keep the RPA at room temperature, or close to body temperature (35° C. to 38° C.) by placing the sample in a body crevice*" (paragraph [0091]). Since RPA uses a recombinase instead of a nickase and enzymes have different temperature requirements and sensitivities, no conclusion can be drawn from document D7 on the issue at hand. Irrespective thereof, it is not apparent why the use of room temperature or body temperature necessarily resulted in a reduced temperature during the amplification process since both systems are relatively stable and moreover allow temperature increases as well as decreases.

29.3 Finally, as regards document D8, the following is relevant. This document discloses a precision heater that is suitable for isothermal amplification reactions. As an example, an isothermal strand displacement amplification is mentioned which has "*an operating temperature and tolerance of ~51°C ± 4°C*" (page 4424, right column, second paragraph), i.e. the assay temperature can cycle around ~51°C within the limits of ± 4°C without negatively affecting the amplification. Figure 2 of document D8 discloses that the specific heater is suitable for this purpose, since its temperature profile remains within these limits.

29.4 Indications that Figure 2 of document D8 disclosed an isothermal amplification reaction are not derivable therefrom, nor has this been argued by the appellant. The appellant, though, argued that since the temperature profile of this heater slightly decreased during the "hold" phase over time, the skilled person using this heater for the methods of documents D2 or D11 would have inevitably arrived at the claimed method.

29.5 It is uncontested that documents D2 and D11 neither mention nor provide pointers for using the specific heater of document D8. Reasons are thus not apparent why the skilled person starting from the method of documents D2 or D11 would have used the heater of document D8. Nor is evidence available that any other heater that might be suitable for the isothermal amplifications of documents D2 or D11 reduces its temperature over time during amplification as specified in claim 1. In these circumstances, it is not convincing that the methods of documents D2 or D11 using a heater would inevitably reduce the temperature during the amplification process.

30. The skilled person would thus not have arrived at the method of claim 1 starting from documents D2 or D11 either alone or in combination with any of the cited prior art documents.

31. The main request thus complies with the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

L. Stridde T. Sommerfeld

Decision electronically authenticated