

Internal distribution code:

- (A) [-] Publication in OJ
- (B) [-] To Chairmen and Members
- (C) [-] To Chairmen
- (D) [X] No distribution

**Datasheet for the decision
of 5 May 2022**

Case Number: T 1655/16 - 3.3.08

Application Number: 10777044.8

Publication Number: 2483422

IPC: C12Q1/68, G01N21/25

Language of the proceedings: EN

Title of invention:

METHOD OF PREPARING A REACTION MIXTURE AND RELATED PRODUCTS

Patent Proprietor:

Thermo Fisher Scientific Baltics UAB

Opponent:

Nederlandsch Octrooibureau N.V.

Headword:

Colored solutions PCR mixture/THERMO FISHER SCIENTIFIC BALTICS

Relevant legal provisions:

EPC Art. 56

RPBA Art. 12(4)

RPBA 2020 Art. 13, 25(2)

Keyword:

Main request - admission (no);

New documentary evidence, new objections and arguments - admission (no);

Auxiliary request 1 - inventive step (yes); fulfils all requirements of the EPC (yes);

Decisions cited:

Catchword:



Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 1655/16 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 5 May 2022

Appellant I: Thermo Fisher Scientific Baltics UAB
(Patent Proprietor) V.A. Graiciuno 8
02241 Vilnius (LT)

Representative: Roques, Sarah E.
J A Kemp LLP
80 Turnmill Street
London EC1M 5QU (GB)

Appellant II: Nederlandsch Octrooibureau N.V.
(Opponent) PO Box 29720
2502 LS The Hague (NL)

Representative: Wall, Leythem
Oxon IP B.V.
Evert Van De Beekstraat 354
1118 CZ Schiphol (NL)

Decision under appeal: **Interlocutory decision of the Opposition
Division of the European Patent Office posted on
6 May 2016 concerning maintenance of the
European Patent No. 2483422 in amended form.**

Composition of the Board:

Chairman B. Stolz
Members: P. Julià
R. Romandini

Summary of Facts and Submissions

- I. European patent 2 483 422 is based on the European patent application no. 10 777 044.8, published under the PCT with the international application number WO 2011/039425. The patent was granted with 15 claims.
- II. An opposition was filed on the grounds set forth in Article 100(a) EPC. The opposition division considered the main request (claims as granted) and auxiliary requests 4 and 5 to lack novelty (Article 54(3) EPC), auxiliary requests 1 to 3 to contravene Article 123(2) EPC, and auxiliary request 6 to fulfill all the requirements of the EPC.
- III. Appeals were lodged by both the patent proprietor and the opponent (appellants I and II, respectively). With the statements setting out their respective grounds of appeal, appellant II filed new documentary evidence and appellant I filed auxiliary requests 1 to 3 and maintained the granted claims as the main request. As an auxiliary measure, both parties requested oral proceedings.
- IV. Appellant I replied to the statement of grounds of appeal of appellant II and requested not to admit the new documentary evidence into the proceedings.
- V. The parties were summoned to oral proceedings. In a communication issued in preparation of the oral proceedings, they were informed of the board's provisional opinion on the issues of the case.
- VI. Both parties replied to the board's communication. Whilst appellant I argued against the admission of the

new documentary evidence into the appeal proceedings, appellant II argued in favour of their admission.

- VII. After several postponements due to, *inter alia*, the COVID-19 pandemic, oral proceedings were rescheduled for 5 May 2022.
- VIII. With submission dated 17 May 2021, appellant II informed the board of the change of representative. With submission dated 13 July 2021, it provided arguments in support of lack of clarity and novelty objections against auxiliary request 3.
- IX. With submission dated 29 March 2022, appellant I withdrew the former main request and auxiliary request 1 and made former auxiliary requests 2 and 3 their main request and auxiliary request 1, respectively.
- X. Oral proceedings were held on 5 May 2022.
- XI. Claims 1 and 4 of the main request read as follows:
- "1. A method of preparing a reaction mixture for Polymerase Chain Reaction (PCR) assay, comprising
- providing a first reagent solution comprising at least one substance required for performing said assay,
 - providing a second reagent solution comprising at least one other substance required for performing said assay,
 - mixing the first and second reagent solutions for providing a mixed solution to be subjected to the PCR process,

characterized in that

- the first reagent solution contains a first colorant providing the first reagent solution a first color,
- the second reagent solution contains a second colorant providing the solution a second color different from the first color,
- said mixing yields a mixed solution having, due to said first and second colorants, a third color different from the first and second colors, and wherein the third color is detected visually."

"4. A solution set for Polymerase Chain Reaction (PCR) assay, comprising

- a first reagent solution comprising at least one substance required for performing said PCR assay,
- a second reagent solution comprising at least one other substance required for performing said PCR assay,

characterized in that

- the first reagent solution is provided with a first colorant having a first color,
- the second reagent solution is provided with a second colorant having a second color different from the first color,
- the first and second reagent solutions are capable of forming, on mixing, a mixed solution having, due to said first and second colorants, a third color different from the first and second colors, wherein the mixed solution comprises fluorescent agent and wherein the absorbance peaks of any of said colorants do not overlap

with the emission or excitation wavelength of said fluorescent agent."

Claims 2 to 3 and claims 5 to 13 are directed to particular embodiments of the method of claim 1 and the solution set of claim 4, respectively. Claim 14 is directed to the use of the method according to any of claims 1 to 3 or the solution set according to any of claims 4 to 13, for preparing a reaction mixture for quantitative PCR.

XII. The auxiliary request 1 contains four claims; claims 1 to 3 read as claims 1 to 3 of the main request and claim 4 is directed to the use of the method according to any of claims 1 to 3, for preparing a reaction mixture for quantitative PCR.

XIII. The following documents are cited in this decision:

- (1): EP-A1-2 239 338 (publication date: 13 October 2010; filing date: 8 April 2010);
- (2): WO-A2-2007/088506 (publication date: 9 August 2007);
- (3): US-A1-2007/0015169 (publication date: 18 January 2007);
- (8): Sanis *et al.*, Handbook of Biological dyes and stains, "Malachite Green", pages 286 and 287;
- (9): Sanis *et al.*, Handbook of Biological dyes and stains, "Methylene Blue", pages 293 to 295;
- (10): "FRET and FLIM techniques" edited by T.W.J. Gadella, Elsevier 2009, page 245.

XIV. The arguments of appellant I, insofar as relevant to the present decision, may be summarised as follows:

Admission of the main request

At the oral proceedings at first instance, the opponent filed a new document that was essential for the opposition division to change its opinion and decide that the claimed subject-matter lacked novelty. The late filing of this document resulted in the patent proprietor not having time to react and consider all new issues raised. The main request was filed with the statement of grounds of appeal and thus, at the earliest opportunity for the patent proprietor to react to this late filed document. The amendment introduced into the main request, namely the subject-matter of granted (dependent) claim 13 into independent claim 4, was straightforward and raised no new issues. This amendment neither contravened Article 123(2) EPC nor Article 84 EPC, because no objections were raised under the former article against the granted claims in the notice of opposition and no objection for lack of clarity could be raised against the granted claims. The filing of the main request in appeal proceedings was not an attempt to start the opposition proceedings anew but a legitimate response to a late filed document; the admission of the main request into the proceedings was in line with the case law.

Admission of new documentary evidence

The opposition division acknowledged the feature "wherein the third color is detected visually" introduced into claim 1 of auxiliary requests 5 and 6 filed at the oral proceedings at first instance, to be

simple and foreseeable in view of the teaching of the entire patent. References to visualising a color and the appearance of a third color were already made at the beginning of and throughout the opposition proceedings. Thus, no late turn of events occurred at the oral proceedings at first instance to justify the admission of new documentary evidence into the appeal proceedings. Nor did appellant II, in the statement setting out their grounds of appeal, refer to any late turn of events at first instance for justifying the filing of the new documentary evidence but only to the relevance of this evidence under Article 56 EPC. According to the case law, the board was not obliged to admit new documents into the appeal proceedings on grounds of their *prima facie* relevance. Moreover, in the present case, none of the new documents was more relevant than other documents on file, in particular document (2), which already addressed the feature introduced into auxiliary requests 5 and 6 filed at the oral proceedings at first instance.

Auxiliary request 1

Admission of the objections of lack of clarity and novelty

In the statement setting out their grounds of appeal, appellant II did not raise any objection under Articles 84 and 54 EPC, the opposition division's decision on Article 54 EPC was not contested and the grounds were based only on Article 56 EPC. Nor did appellant II reply to appellant I's statement of grounds of appeal. The objections under Articles 84 and 54 EPC were raised at a late stage of the appeal proceedings, once the parties were informed of the board's opinion on the issues of the case. Their introduction at this late stage of the proceedings was

an amendment of appellant II's case which had thus to be justified and their admission into the appeal proceedings was subject to the board's discretion (Article 13(1) RPBA). According to Article 13(2) RPBA, after notification of a summons to oral proceedings, an amendment to a party's case could not be taken into account unless there were exceptional circumstances, which had been justified with cogent reasons by the party concerned. In the present case, appellant II failed to provide any reasons that could justify the introduction of the late filed objections and lines of attacks under Articles 84 and 54 EPC; no exceptional circumstances had been put forward, let alone justified by cogent reasons.

Article 56 EPC

The closest prior art document (2) referred to the difficulties facing the application of PCR on a large scale, in particular when preparing PCR reagents and amplification mixture. In order to overcome them, document (2) disclosed the use of an inert thermally controllable polymer and the preparation of a ready-to-use master mix in form of a gel, wherein said inert polymer and all or part of the PCR reagents were pre-aliquoted in appropriate quantities. In one embodiment, the master mix comprised the inert polymer, PCR salts and buffers, oligonucleotide primers, the polymerase enzyme necessary for PCR amplification of the target nucleic acid, and one or more inert dyes. There was neither a reference to, nor a discussion on, the purpose, function or effect of the inert dyes; document (2) suggested only that different dyes could be used to distinguish different master mixes for amplification of different target nucleic acids. However, since the master mix had already all PCR

reagents, the use of these inert dyes had nothing to do with, and was unrelated to, the monitoring of the addition of further PCR reagents. Moreover, the addition of (liquid) solutions with further PCR reagents and yet another dye, required to disregard the fact that the master mixes disclosed in document (2) were in the form of a gel.

There was neither a pointer nor a motivation in document (2) that would have led a skilled person to document (3), the combination of these documents required hindsight knowledge of the patent. Even if a skilled person would have combined these documents, this combination did not render the claimed subject-matter obvious. The purpose of the dyes disclosed in document (3) were not to monitor the addition of PCR reagents but to ensure a complete bacterial lysis and thereby optimise the method for preparing plasmid DNA. Moreover, the change of color disclosed in document (3) did not result from the addition of a further dye but from a pH change of the solution (which allowed to measure and detect the efficiency of the lysis). Documents (2) and (3) addressed different technical problems (addition of PCR reagents vs. measuring/ detecting the efficiency of lysis) that were overcome by different solutions (preparation of a pre-aliquoted master mix vs. use of a pH-dependent change of color). The combination of these documents was not obvious, required hindsight knowledge of the patent, and did not render the claimed subject-matter obvious.

- XV. The arguments of appellant II, insofar as relevant to the present decision, may be summarised as follows:

Admission of the main request

The main request could and should have been filed at first instance. The patent proprietor was given ample opportunity to file new requests at first instance, including at the oral proceedings, where two new auxiliary requests were filed and admitted into the proceedings. The features introduced into all auxiliary requests filed at first instance were taken from the description of the patent, in none of them was the subject-matter of a dependent claim introduced into an independent claim, this was only done at a late stage in appeal proceedings. The document filed at the oral proceedings at first instance was merely evidence of a skilled person's common general knowledge, it was neither essential nor changed the opposition division's decision on lack of novelty over document (1); document (1) was novelty destroying regardless of that new document. The filing of the new main request was an attempt to start the opposition proceedings anew and thus, its admission in appeal proceedings was not in line with the case law.

Admission of new documentary evidence

The filing of the new documentary evidence in appeal proceedings was a reaction to a late turn of events at the oral proceedings at first instance, namely the late filing of auxiliary requests 5 and 6 with the feature "wherein the third color is detected visually" in claim 1; a feature taken from the description of the patent and not present in any of the granted claims. The opposition division erred in considering this feature to be simple, foreseeable and not substantial; this was shown by the fact that claim 1 of these late filed auxiliary requests was considered to overcome a novelty objection over document (1). This feature was substantial, not foreseeable, and resulted in a new

situation compared to that of the granted claims. The opponent had not been allowed to respond accordingly to the late filing at first instance of auxiliary requests with this surprising amendment and feature. The new documentary evidence was filed at the first opportunity after the oral proceedings at first instance. According to the case law, a late turn of events was a sound and plausible reason for the admission of new documents into the appeal proceedings. Thus, the admission of the new documentary evidence into the appeal proceedings was a legitimate response to a late filed and unpredictable amendment of the claims by introduction of a feature taken from the description of the patent. The new documentary evidence was highly relevant and directly addressed the feature introduced into claim 1 of the main request in appeal.

Auxiliary request 1

Admission of the objections of lack of clarity and novelty

Although the objections under Articles 84 and 54 EPC were based on late filed arguments, the objection of lack of novelty over document (1) was examined and decided by the opposition division in the context of claim 1 of auxiliary request 5 at first instance which was identical to claim 1 of auxiliary request 1 in appeal. The reasons given by the opposition division in the decision under appeal for acknowledging novelty of claim 1 of auxiliary request 5 at first instance had always been on file and were thus part of the appeal proceedings. Therefore, although the arguments on lack of novelty and clarity were late filed, they did not change the facts of the case. The objection of lack of clarity was directly derivable from appellant I's arguments on, and the interpretation of, the feature

"detected visually" made in the discussion under Article 54 EPC.

According to the case law, late filed arguments and lines of attack could be admitted into the proceedings if they were fundamental and could not be ignored since any discussion on inventive step was rendered meaningless if they were left unresolved. Regardless of Article 13(1) RPBA, the boards had no discretion when it came to the admissibility of late filed arguments based on facts already in the proceedings, Article 114(2) EPC did not provide a justification for such a discretion. In the present case, the new arguments on Articles 84 and 54 EPC were fundamental and could not be left unresolved before a discussion on Article 56 EPC. Moreover, they were based on identical factual and evidential framework (same passages of document (1) and the PCT patent application) to that already cited in the proceedings; the board had thus no discretion for not admitting them into the proceedings.

Article 56 EPC

The closest prior art document (2) referred to the problems associated with the application of PCR on a large scale and, for overcoming them, disclosed the use of a master mix comprising all or part of the reagents required for PCR amplification. In line therewith, claim 1 of document (2) was directed to a master mix comprising only part of the PCR reagents and dependent claims 2 and 10 to the oligonucleotide primers required for amplification of the target nucleic acid and to an effective amount of one or more inert dyes. The preferred dyes (malachite green, methylene blue) were visual colors (absorption wavelengths in documents (8) and (9)) and the use and combination of several dyes

were explicitly envisaged in document (2); the mixing of these dyes inherently led to a third color. There was no indication as regards the purpose or function of these dyes and thus, neither a restriction nor a limitation to any particular purpose or function could be derived from document (2). The method disclosed in this document differed from the claimed method in that there was no reference to a third color (resulting from mixing a first and second color; even though a third color was inherently obtained by following the teachings of document (2)), nor to a visual detection of said third color.

Starting therefrom, the objective technical problem to be solved was the provision of a method for monitoring the addition of further PCR reagents to a master mix and for the detection of errors during such addition, i.e. the problem identified in paragraph [0021] of the patent. The proposed solution of this problem, i.e. the claimed method, was obvious in light of document (3).

Although document (3) did not address the preparation of PCR mixtures, the nucleic acid isolated according to the method disclosed in this document was intended to be used in a PCR reaction. Thus, no hindsight was required to combine documents (2) and (3). Document (3) referred also to the problems associated with an inefficient sample handling, in particular those related to buffer pipetting. In order to prevent them and avoid accidental errors, document (3) taught the use of one or more dyes - and the examination of color changes by eye or by an improved visual recognition - for assessing whether the performed manipulations were complete and successful. In Example 1, the teachings of document (3) were exemplified by using several colors. Regardless of the specificities of this example, the

concept disclosed in document (3) was generally applicable. Whilst the monitoring of the addition of further reagents and the detection of errors in document (3) was not carried out in the context of a PCR reaction, no technical prejudice hindered or prevented a skilled person from applying the solution disclosed in document (3) when facing the same technical problem as that identified in document (2). Although in different fields (PCR amplification vs. cell lysis), documents (2) and (3) faced the same problem (difficulties in buffer/reagent pipetting and error detection) and shared a common principle or concept (visual color recognition). Thus, starting from the closest prior art document (2), the skilled person would have been motivated to consult the disclosure of document (3) and the combination of these two documents rendered the claimed subject-matter obvious.

XVI. The appellant I (patent proprietor) requested that the decision under appeal be set aside and the patent be maintained in amended form on the basis of the main request or, in the alternative, of auxiliary request 1.

XVII. The appellant II (opponent) requested that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Admission of the main request

1. The main request was originally filed as auxiliary request 2 with appellant I's statement of grounds of appeal. Claims 1 to 3 of the main request are directed to a method of preparing a reaction mixture for PCR assay which is identical to the method of claims 1 to 3 of auxiliary requests 5 and 6 at first instance, the

latter request being that upheld by the opposition division. Claims 4 to 13 of the main request are directed to a solution set for PCR assay, wherein the feature in claim 4 "... the mixed solution comprises fluorescent agent and ... the absorbance peaks of any of said colorants do not overlap with the emission or excitation wavelength of said fluorescent agent" is the subject-matter of granted claim 13.

2. The patent proprietor maintained the granted claims as the main request throughout the whole proceedings at first instance and filed several auxiliary requests in response to the opponent's notice of opposition (auxiliary requests A to D) and the opposition division's preliminary opinion (auxiliary requests 1 to 4). At the oral proceedings at first instance, the opposition division admitted into the proceedings a new auxiliary request 4 to replace former auxiliary request 4 as well as new auxiliary requests 5 and 6 (the latter request being that upheld by the opposition division).
3. In all these auxiliary requests, amendments were introduced into claims 1 and 4. However, none of these auxiliary requests contained the subject-matter of granted claim 13. Although the amendments introduced into claim 4 of these auxiliary requests were mainly subject-matter taken from the description of the patent, claim 4 of some of these requests (auxiliary request D, former and new auxiliary request 4, and auxiliary request 5) was amended by introducing the subject-matter of granted claim 9, although slightly modified. Whilst the feature introduced into claim 4 of these auxiliary requests reads "... wherein the concentration of said colorants in respective reagent solutions corresponds to absorbances of the said

solutions at their maximum absorption wavelength of 0.001-0.5 at 1 mm light path, when diluted to the desired PCR processing concentration", claim 9 as granted further refers to the preferred ranges "in particular 0.01 - 0.5, preferably 0.03 - 0.15".

4. The patent proprietor had thus ample opportunity to file claim requests during the first instance proceedings, including at the oral proceedings before the opposition division. The patent proprietor made use of these opportunities and filed several auxiliary requests, wherein amendments introduced into the independent claims were subject-matter taken from the description of the patent as well as from the granted claims. In view thereof, the board considers that the main request with the amendment introduced into claim 4 (subject-matter of granted claim 13) could and should have been filed at the first instance proceedings.

5. Appellant I argues that the filing of the main request in appeal proceedings was the first opportunity for the patent proprietor to react to the late filing of document (10) at the oral proceedings at first instance and the admission by the opposition division of this late filed document into the proceedings. The board does not agree therewith.
 - 5.1 Document (10) was filed at the beginning of the oral proceedings at first instance during the discussion of whether the granted claims fulfilled the requirements of Article 54(3) EPC (cf. page 1, point 2.2, of the minutes of the oral proceedings at first instance; page 6, point 1, of the decision under appeal). Document (10) discloses the absorption maximum and extinction coefficients (ϵ) of the most commonly used quenchers, including Dabsyl (Table 6.2), a quencher

referred to in document (1), the document cited under Article 54(3) EPC in the decision under appeal (cf. page 6, point 2, of the decision under appeal).

5.2 The opposition division stated that the disclosure of document (10) "was essential for the assessment of novelty over D1 in particular for a complete appreciation of the disclosure of Example 1 therein". At the same time, the document was considered to reflect the skilled person's general common knowledge in the field of fluorescent dyes and quenchers (cf. page 1, point 2.2, of the minutes of the oral proceedings at first instance; page 6, point 1.3, of the decision under appeal). The board agrees with this assessment. The nature of document (10) falls within the notion of common general knowledge as developed by the case law (cf. "Case Law of the Boards of Appeal of the EPO", 9th edition 2019, I.C.2.8.1, 77); the document provides only evidence of the skilled person's common general knowledge when reading the prior art, in particular Example 1 of document (1). The filing and content of document (10) did not raise any new facts or issues. Moreover, the patent proprietor was also given the opportunity to file new auxiliary requests once document (10) had already been admitted into the proceedings. As shown above, the patent proprietor had replaced auxiliary request 4 and filed new auxiliary requests 5 and 6.

5.3 In light of the course of events at first instance and the nature and disclosure of document (10), the board considers that the filing of the main request in appeal proceedings is an attempt to re-open the opposition proceedings, which is not in line with the purpose of an appeal proceedings as defined in the case law (cf. "Case Law", *supra*, V.A.1.1, 1133).

6. Thus, the board, in the exercise of its discretion (Article 25(2) RPBA 2020 and Article 12(4) RPBA 2007), decides not to admit the main request into the appeal proceedings.

Admission of new documentary evidence

7. Claim 1 of the main request is identical to claim 1 of auxiliary requests 5 and 6 filed at oral proceedings at first instance. The opposition division considered the feature introduced into claim 1, namely "wherein the third color is visually detected", to be "simple enough and also foreseeable in view of the teaching of the entire patent for the opponent not to be surprised or confronted with major difficulties" (cf. page 15, point 12.3, of the decision under appeal). The introduction of this feature was also considered to overcome the novelty objection over document (1) (cf. page 17, point 14.3, of the decision under appeal).
8. According to the minutes of the oral proceedings at first instance, the admission of auxiliary request 5 into the proceedings was discussed with the parties and, after a short break, the request was admitted into the proceedings (cf. page 2, point 5.1, of the minutes of the oral proceedings). The decision under appeal refers to both the opponent's reasons against the admission of this auxiliary request and those given by the opposition division for the admission of auxiliary request 5 into the proceedings (cf. page 15, points 12.2 and 12.3, of the decision under appeal).
9. According to the decision under appeal, the opponent referred only to the fact that auxiliary request 5 "was filed late and could have been provided earlier" (cf.

page 15, point 12.2, of the decision under appeal). Neither in the minutes of these oral proceedings nor in the decision under appeal, there is any reference to the opponent arguing that the feature introduced into claim 1 of auxiliary request 5 resulted in a turn of events that required an interruption or postponement of the oral proceedings for the opponent to carry out a new search of prior art documents.

10. Appellant II's argument for the introduction of the new documentary evidence into the appeal proceedings based on the relevance of the feature introduced into claim 1 of auxiliary request 5 at first instance (main request in appeal), was put forward at a late stage of the appeal proceedings, namely in response to the board's provisional opinion not to admit this evidence into the appeal proceedings. In the statement of grounds of appeal, appellant II stated only that the new documents "are submitted on time as they are *prima facie* relevant and they are filed to demonstrate that the decision taken by the OD on inventive step was not correct. All these documents were therefore filed at the earliest possible time ... and therefore admissible". There was thus a reference only to the relevance of this new documentary evidence in the light of the opposition division's decision on Article 56 EPC.
11. The board agrees with appellant II that the relevance of the new documentary evidence must always be taken into account. However, in line with the case law, the relevance of new evidence cannot be the sole, decisive reason for a board to admit new evidence into the proceedings; the specific circumstances and particular course of events of the proceedings at first instance must also be taken into account (cf. "Case Law", *supra*, V.A.4.5.2, 1216). In the present case, several problem

and solution approaches - based on the new documentary evidence - are formulated by appellant II as possible alternatives to the approach formulated by the opposition division in the decision under appeal and based on the combination of the closest prior art document (2) with document (3). Appellant II failed to provide any convincing reasons why the new documentary evidence and the alternative approaches based thereupon could not have been filed at an earlier stage of the proceedings at first instance.

12. The relevance of the feature introduced into claim 1 of auxiliary request 5 at first instance (main request in appeal) is derivable from the numerous references throughout the whole description of the patent, such as "one can tell directly by the color of the solution, whether it is the first reagent solution, the second reagent solution or the mixture of these" (cf. paragraph [0024]), "the inspection of the color can be made visually or by automatic optical means" (cf. paragraph [0026]), "the term "color" herein means any detectable spectral response (of a solution) to white light in the visual range ... which provides a colored visual appearance for the solution ... the colors are distinguishable, preferably by the naked eye ... capable of giving the solution a perceivable color" (cf. paragraphs [0050] to [0052], [0064], [0065], [0067] and [0093]), as well as by the examples of the patent, wherein visual colors are exemplified (cf. paragraph [0104] and [0120]).

13. Moreover, although the feature introduced into claim 1 of auxiliary request 5 at first instance (main request in appeal) was not present in any of the auxiliary requests filed in the written phase of the opposition proceedings, references were made throughout these

proceedings to the "appearance" and the detection of a third color resulting from the mixing of first and second reagent solutions containing first and second colors (cf. page 3, point 2.1, and page 4, point 3.2, of the opposition division's preliminary opinion).

14. Thus, in view of the whole content of the patent and the course of events at first instance, the filing of auxiliary request 5 at oral proceedings at first instance with claim 1 amended by the introduction of the feature "the third color is detected visually" neither amounted to, nor can be seen as, a late turn of events requiring a search of new prior art documents and the interruption or postponement of these oral proceedings at first instance. Neither the former nor the latter were ever requested by the opponent at first instance. Therefore, the board considers that the first opportunity for the appellant II to react to the introduction of the feature "the third color is detected visually" was not in appeal proceedings but at an earlier stage of the proceedings at first instance.
15. Thus, the board, in the exercise of its discretion (Article 25(2) RPBA 2020 and Article 12(4) RPBA 2007), decided not to admit the new documentary evidence into the appeal proceedings.

Auxiliary request 1

Admission of the objections of lack of clarity and novelty

16. Claim 1 of auxiliary request 1 is identical to claim 1 of auxiliary requests 5 and 6 filed at the oral proceedings at first instance (the latter request being that upheld by the opposition division). The opposition division considered the subject-matter of this claim to be novel over document (1) (Article 54(3) EPC) (cf.

page 17, point 14.3, of the decision under appeal; page 3, point 5.4, of the minutes of the oral proceedings at first instance). According to the minutes of the oral proceedings at first instance, after discussing the admissibility of auxiliary request 5 and the requirements of Article 123(2) EPC, the opponent did not raise any objection under Article 84 EPC and none was raised by the opposition division (cf. page 3, point 5.3, of the minutes of the oral proceedings at first instance). Since auxiliary request 6 was based on claims 1 to 3 and 15 of auxiliary request 5, auxiliary request 6 was considered to satisfy the requirements of Articles 123(2), 84 and 54 EPC without further discussion (cf. page 3, point 6.2, of the minutes of the oral proceedings at first instance). In line therewith, there is no reference to Article 84 EPC as regards auxiliary requests 5 and 6 in the decision under appeal.

17. In the statement of grounds of appeal, the sole decision of the opposition division contested by appellant II was the decision taken on Article 56 EPC. There was no reference to any other article of the EPC nor to document (1), the sole document cited under Article 54(3) EPC in the decision under appeal. Nor did appellant II refer to any of Articles 84 and 54 EPC in response to the board's communication informing the parties of the board's provisional opinion on the issues of the case. Appellant II only argued therein on the reasons for the admission of the new documentary evidence filed in the appeal proceedings.

18. It was only after informing the board of a change of representative that appellant II filed a submission with objections under Articles 84 and 54 EPC and new lines of attack against the requests on file. The

introduction of these new objections and lines of attack was justified by appellant II by reference to the comments and amendments made by appellant I in the statement of grounds of appeal and concerning former auxiliary request 1. No other reasons were given by appellant II to explain the introduction of the new objections and lines of attack at that late stage of the appeal proceedings.

19. The introduction of the new objections under Articles 84 and 54 EPC and the new lines of attack are an amendment of appellant II's appeal case within the meaning of Article 13 RPBA. Thus, in line therewith, these new objections and lines of attack are not to be taken into account unless there are exceptional circumstances justified by cogent reasons.
20. In light of the course of events described above, the board fails to see any exceptional circumstances that may justify the admission of the new objections and lines of attack under Articles 84 and 54 EPC. Appellant I's comments and amendments referred to by appellant II were made at the earliest stage of the appeal proceedings and appellant II had ample opportunity to put forward the new objections and lines of attack at an earlier stage of these proceedings. The reasons given by appellant II are neither cogent nor justify the admission of the new objections and lines of attack at such late stage of the appeal proceedings.
21. The board also disagrees with appellant II's argument that the new objections and lines of attack are fundamental and based on a factual and evidential framework identical to that already present in the appeal proceedings. The framework of appeal proceedings is not only set out and defined by the decision under

appeal alone but also by the reasons and evidence provided in the statement of grounds of appeal. As stated above, the purpose of appeal proceedings is not to re-open and continue the opposition proceedings but to review the decision of the opposition division (cf. "Case Law", *supra*, V.A.1.1, 1133). Appellant II's new objections and lines of attack under Articles 84 and 54 EPC are nothing more than an attempt to re-open the opposition proceedings and thus, not admissible.

22. Thus, the board, in the exercise of its discretion (Article 13 RPBA 2020), did not admit appellant II's new objections and lines of attack under Articles 84 and 54 EPC into the appeal proceedings.

Article 56 EPC

23. The closest prior art document (2) relates to the same technical field as the patent, namely PCR amplification of nucleic acids, and refers to several problems of the PCR method, in particular those due to the evaporation of the PCR solution and those arising from the application of PCR on a large scale (cf. page 3, third paragraph, to page 4, first paragraph). In this context, reference is made to the fact that specialised staff is required and that "the operator must ... prepare in advance all the reagents necessary for setting up the PCR reaction and aliquot into each of the wells of the plate the correct volume of amplification mixture, with all the risks deriving from the variability due to the operator" (cf. paragraph bridging pages 3 and 4).
24. As a solution to these problems, document (2) discloses the preparation of a master mix which is "mixed with an effective amount of an inert temperature controllable

polymer" and which may comprise "all or part of the reagents required for the PCR amplification in the appropriate quantities" (cf. page 4, second and third paragraphs; claim 1). In one embodiment, the "master mix also comprises an effective amount of one or more inert dyes, preferably methylene blue or malachite green" (cf. page 6, fourth paragraph; claim 10). The teachings of document (2) are exemplified by the "reference dye (ROX)" (cf. page 9), known in the art as the inert fluorescent dye carboxyrhodamine.

25. Document (2) states that the amplification of a plurality of different target nucleotide sequences using a different dye for each target nucleic acid sequence "can be envisaged" (cf. paragraph bridging pages 6 and 7). However, there is no reference to a mixing of first and second reagent solutions with first and second colorants, respectively, and to the appearance of a third color - due to the mixing of said first and second colorants, which is different from those of the first and second colorants and which is detected visually, let alone a reference to the use of such a mixed solution as a reaction mixture for a PCR assay.

26. Starting therefrom, the objective technical problem is formulated as the provision of an alternative method for avoiding errors in the preparation and aliquoting of reaction mixtures for PCR amplification assay. There is no reference in document (2) to the detection of these errors. Consequently said detection cannot be part of the problem to be solved, otherwise hindsight knowledge of the patent would be implied.

The claimed method solves this problem and further allows the detection of errors, an effect that results from the proposed solution.

27. As stated above, document (2) refers to the presence of one or more inert dyes in the master mix (cf. page 6, fourth paragraph). An inert dye, such as the exemplified fluorescent dye ROX, is known in the field to be an internal passive reference which is not affected by PCR amplification and which allows for a normalisation of the PCR data. There is no hint or pointer in document (2) that could lead a skilled person to use first and second PCR reagent solutions with first and second colorants that, when mixing, result in a PCR reaction (master) mixture of a third color different from those of the first and second colorants and which is detected visually. Hindsight knowledge of the patent is required for a skilled person to envisage such a use from the disclosure of document (2) alone. Likewise, hindsight knowledge of the patent is required for combining the disclosure of document (2) with that of document (3).
28. Document (3) is not concerned with PCR amplification but with the isolation of nucleic acids, in particular plasmid DNA from *E. coli* and other organisms (cf. *inter alia*, abstract, paragraph [0003]). It is only after carrying out the disclosed purification method that the isolated pure plasmid DNA may be used, *inter alia*, in a PCR assay (cf. paragraphs [0005] and [0023]). Thus, it is arguable whether documents (2) and (3) are indeed in the same technical field so that a skilled person, when starting from the former, would be actually aware of the teachings of the latter document.

29. Moreover, whilst document (3) refers to the problems associated with inefficient sample handling related to pipetting (cf. paragraph [0011], penultimate sentence; paragraph [0012]), the disclosure of this document is engaged and deals with the lysis of *E. coli* and other bacterial organisms, in particular a modified mild alkaline lysis, which is an earlier step of the method for isolating plasmid DNA disclosed in document (3) (cf. *inter alia*, paragraphs [0006], [0007], [0031], [0047]; claims 1 and 8).
- 29.1 The contribution of document (3) to the art is the "addition of indicator dyes to the alkaline lysis based buffers [which] allows for easy and rapid visual monitoring of the resuspension, lysis, and neutralization steps. The ability to assess the process increases plasmid yield and also reduces errors" (cf. paragraph [0003]). As stated in document (3), an "improved visual recognition of lysis" or a "visual monitoring" of the modified mild alkaline lysis improves the "overall quality and yield" and "increases efficiency and significantly reduces the time required to process each sample" (cf. *inter alia*, paragraph [0011], last sentence; paragraphs [0012], [0023] and [0031]).
- 29.2 Although document (3) refers to indicator dyes that are "different in each of the buffers and may not undergo a response to pH", in the preferred embodiments "the color change is based on indicator dyes whose color is related to the pH of the solution" (cf. paragraph [0012]), and the preferred dyes "are ones that display different color ranges in the pH range suitable for plasmid DNA isolation" (cf. paragraph [0013]; claims 2 and 9).

- 29.3 Indeed, the colors of the indicator dyes present in the three buffers exemplifying the teachings of document (3) undergo a response to pH, namely buffer P1 (Resuspension buffer - with Phenol red, around neutral pH 7.0-8.0, red in color), buffer P2 (Lysis buffer - with Emerald Green, basic pH, blue-green in color) and buffer P3 (Neutralisation buffer - with Phenol Red, acidic pH, yellow in color). It is worth noting here that the same colorant is used in buffers P1 and P3 (cf. paragraphs [0013], [0016] to [0018], [0027], and [0033] to [0045]). The color changes reported in Example 1 of document (3) are based on these indicator dyes which are related to the pH and thus reflect the pH of the solutions after mixing. In Example 1, the addition of buffer P2 (basic pH, blue-green color) to buffer P1 (neutral pH, red color) results in a solution of "a deeper red in color" (cf. paragraphs [0051] and [0052]), and the addition of buffer P3 (acidic pH, yellow color) to the latter solution results in a final solution that "becomes yellow" (cf. paragraph [0053]).
- 29.4 It is questionable whether a skilled person would have considered the preferred strategies described and exemplified in document (3) for visual monitoring of a modified mild alkaline lysis of *E. coli* host cells based on pH dependent colorants for solving the objective technical problem as formulated above and arriving thereby at the claimed subject matter. The board agrees with the decision of the opposition division and considers that this question must be answered in the negative (cf. page 20, point 16.4, of the decision under appeal).
30. Therefore, the skilled person would not have combined the teaching of documents (2) and (3) and, even if they were combined, hindsight knowledge of the patent

would have been required for a skilled person to arrive at the method claimed in auxiliary request 1.

31. Thus, auxiliary request 1 fulfils the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar:

The Chairman:



L. Malécot-Grob

B. Stolz

Decision electronically authenticated