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Datasheet for the decision of 13 March 2018

Case Number: T 1739/13 - 3.3.08

Application Number: 01274041.1

Publication Number: 1354036

IPC: C12N15/10, C12Q1/68

Language of the proceedings: EN

Title of invention:
COMPOSITIONS, METHODS, AND KITS FOR ISOLATING NUCLEIC ACIDS USING SURFACTANTS AND PROTEASES

Patent Proprietor:
Life Technologies Corporation

Opponent:
KÖNIG SZYNKA TILMANN VON RENESSE

Headword:
Isolation nucleic acids surfactants/LIFE TECHNOLOGIES

Relevant legal provisions:
EPC Art. 123(2)
RPBA Art. 12(4), 13(1), 13(3)

Keyword:
Main request - admission into the appeal proceedings (no)
Auxiliary Request 1 - added subject-matter (yes)
Auxiliary requests 2-13 - admission into the appeal proceedings (no)/added subject-matter (yes)

Decisions cited:
T 0190/99, T 0457/02, T 0361/08, T 0679/09, T 2487/12, T 0389/13, T 0782/16

Catchword:

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Case Number: T 1739/13 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 13 March 2018

Appellant: (Patent Proprietor)	Life Technologies Corporation 5823 Newton Drive Carlsbad, CA 92008 (US)
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Representative:	Roth, Carla König-Szynka-Tilmann-von Renesse Patentanwälte Partnerschaft mbB Postfach 11 09 46 40509 Düsseldorf (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 10 June 2013 revoking European patent No. 1354036 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman B. Stolz
Members: P. Julià
D. Rogers

Summary of Facts and Submissions

I. European patent no. 1 354 036 was based on European patent application no. 01 274 041.1, filed under the Patent Cooperation Treaty and published as WO 02/090539 (hereinafter "the patent application"). The patent application contained 64 claims with four independent claims, claims 1, 25, 41 and 64 which read as follows (emphasis by the board):

"1. A method for obtaining nucleic acid from a biological sample and binding the nucleic acid to a solid phase, comprising:
contacting the biological sample with a disrupting buffer, wherein the disrupting buffer comprises:
a protease; and
a cationic surfactant;
substantially neutralizing the cationic surfactant; and
binding the nucleic acid to a solid phase."

"25. A method for obtaining nucleic acid from a biological sample and binding the nucleic acid to a solid phase, comprising:
contacting the biological sample with a disrupting buffer, wherein the disrupting buffer comprises:
a protease; and
a cationic surfactant; and
binding the nucleic acid to a solid phase."

"41. A kit comprising:
a protease;
a cationic surfactant; and
a second surfactant, wherein the second surfactant
substantially neutralizes the cationic surfactant."

"64. A kit for obtaining nucleic acid from a biological sample comprising:
a protease;
a cationic surfactant;
a non-ionic surfactant, wherein the non-ionic surfactant **permits the binding** of nucleic acid to a solid phase in the presence of the protease and cationic surfactant; and
a buffer with a high salt concentration."

Claims 2 to 24 and 26 to 40 were directed to preferred embodiments of claims 1 and 25, respectively. Claims 42 to 63 were directed to preferred embodiments of claim 41.

II. The patent was granted and published with forty five claims with three independent claims, claims 1, 23 and 45 which read as follows (emphasis by the board):

"1. A method for obtaining nucleic acid from a biological sample and binding the nucleic add [sic] to a solid phase, comprising the steps:
(a) contacting the biological sample with a disrupting buffer, wherein the disrupting buffer comprises:
a protease; and
a cationic surfactant;
(b) adding a second non-ionic surfactant wherein the non-ionic surfactant **permits the binding** of nucleic acid to a solid phase in the presence of the protease and cationic surfactant and a buffer with high salt concentration
(c) binding the nucleic add [sic] to a solid phase."

"23. A kit comprising:
a protease;
a cationic surfactant; and

a second non-ionic surfactant, wherein the non-ionic surfactant **permits the binding** of nucleic acid to a solid phase in the presence of the protease and cationic surfactant."

"45. A kit for obtaining nucleic acid from a biological sample comprising:
a protease;
a cationic surfactant;
a non-ionic surfactant, wherein the non-ionic surfactant **permits the binding** of nucleic acid to a solid phase in the presence, of the protease and cationic surfactant; and
a buffer with a high salt concentration."

Claims 2 to 22 and claims 24 to 44 were directed to preferred embodiments of claims 1 and 23, respectively.

III. An opposition to the grant of the patent was filed relying on the grounds for opposition under Articles 100(a) and 100(c) EPC. In a decision under Article 101(3)(b) EPC, the opposition division revoked the patent because none of the requests filed at oral proceedings fulfilled the requirements of the EPC; the main request and auxiliary request 3 contravened Article 84 EPC, auxiliary request 1 did not fulfil the requirements of Article 56 EPC and auxiliary request 2 did not fulfill those of Articles 84 and 123(2) EPC.

The main request had 24 claims with two independent claims, claims 1 and 22. Claim 1 read as granted claim 1, except for the presence of a comma in step (b) and the correction of a clerical error in the preamble and in step (c) (emphasis by the board):

"1. ... and binding the nucleic **acid** to ...

(b) ... the binding of nucleic acid to a solid phase in the presence of the protease and cationic surfactant, and a buffer with high salt concentration
(c) binding the nucleic **acid** to a solid phase."

Claim 22 read as granted claim 23 with a combination of dependent granted claims 30 and 31 (requiring the presence of a ribonuclease inhibitor and defining the nature of said ribonuclease inhibitor, respectively):

"... [as granted claim 23] ... ; and a ribonuclease inhibitor,

wherein the ribonuclease inhibitor is selected from at least one of the group comprising vanadylate ribonucleoside complexes, phenylglyoxal, p-hydroxyphenylglyoxal, polyamines, spermidine, 9-aminoacridine, iodoacetate, bentonite, poly[2'-O-(2,4-dinitrophenyl)]poly(adenyhtic acid), zinc sulfate, bromopyruvate, formamide, copper, and zinc."

Auxiliary requests 1 and 2 had 22 claims. Claim 1 of these auxiliary requests read as claim 1 of the main request. Claim 22 of auxiliary request 1 read as granted claim 23 with the additional feature:

"... [as granted claim 23] ... ; wherein the kit further comprises a ribonuclease inhibitor."

Claim 22 of auxiliary request 2 read as follows:

"22. A kit comprising:
a disrupting buffer consisting of a protease and a cationic surfactant;

and a second non-ionic surfactant, wherein the non-ionic surfactant permits the binding of nucleic acid to a solid phase in the presence of the protease and cationic surfactant;
wherein the kit further comprises a ribonuclease inhibitor."

Auxiliary request 3 had 21 claims, none of them directed to a kit. Claim 1 was the sole independent claim and read as claim 1 of the main request, except for the substitution of the term "comprising" by "consisting of" in the preamble.

IV. An appeal was lodged by the patent proprietor (appellant). With the statement setting out the grounds of appeal, the appellant filed a new main request and new auxiliary requests 1 to 6.

V. The opponent (respondent) replied to the statement of grounds of appeal.

VI. Both parties requested oral proceedings as an auxiliary measure.

VII. The board summoned the parties to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), the board expressed a provisional, non-binding opinion on some of the issues of the case. In particular, the board was minded not to admit any of appellant's claim requests into the proceedings because, *inter alia*, when compared with all requests underlying the decision under appeal, none of the requests filed with the grounds of appeal contained a comma in step (b) of claim 1.

VIII. Both parties replied to the board's communication. In its reply, the appellant filed a new main request and new auxiliary requests 1 to 6, and its former main request and auxiliary requests 1 to 6 were refiled as auxiliary requests 7 to 13.

IX. The independent claims 1 and 22 of the **main request** and of the **auxiliary request 1** are identical to claims 1 and 22 of the main request underlying the decision under appeal, except that claim 22 does not specify the nature of the ribonuclease inhibitor. The main request further comprises claims 23 to 25 which are dependent on claim 22 and define the nature of the ribonuclease inhibitor (granted claims 31 to 33). Auxiliary request 1 does not contain any claim dependent on claim 22.

X. Oral proceedings were held on 13 March 2018 in the presence of both parties.

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

Admission of the main request

The main request fulfilled the conditions for admitting amended requests in appeal proceedings after the arrangement of oral proceedings. The introduction of a comma in step (b) of claim 1 was a direct response to the board's communication; the scope of the discussion was not changed since the respondent itself had raised the issue in its reply to the statement of grounds of appeal, and the amendment did not result in a delay of the proceedings.

The statement of grounds of appeal showed that appellant's intention had always been to defend claim 1 unamended, as it was before the opposition division. The absence of the comma in step (b) of claim 1 of the requests filed with the grounds of appeal was an evident error. In view thereof, there was no need to file new requests for addressing this issue in response to respondent's reply to the grounds of appeal. The Minutes of the oral proceedings before the opposition division mentioned a discussion about a semicolon in step (b) of claim 1 of a former main request but not a lengthy discussion on the relevance of a comma. The semicolon was replaced by a comma, the former main request was withdrawn and the amended request was the main request underlying the decision under appeal. This issue was not relevant in the decision under appeal and, accordingly, not discussed therein.

The opposition division considered the main request before it to contravene Article 84 EPC due to the definition of the nature of the ribonuclease inhibitor in independent claim 22 and the presence of inhibitors of yet another nature in dependent claims 23 and 24. The amendments introduced into claims 22 to 25 of the main request in appeal were a serious attempt to overcome this objection and did not raise new issues. The scope of claim 22 in appeal was identical to that of claim 22 of the auxiliary request 1 at first instance.

Admission of the auxiliary request 1

The reasons given for the main request to explain the introduction of a comma in step (b) of claim 1 equally applied to auxiliary request 1. Although there were minor differences between claim 22 of auxiliary request

1 in appeal and at first instance, they were clerical in nature and did not change the scope of the claim nor its technical meaning.

Auxiliary request 1
Article 123(2) EPC

The method of claim 1 of auxiliary request 1 was directly derivable from the combination of claims 1, 4 and 9 of the patent application.

The features present in claim 1 were not picked out of their original context in the patent application but were directly and unambiguously derivable from the patent application, even though in an implicit manner.

From the disclosure of the patent application, a skilled person would have immediately understood that the cationic surfactant in the disrupting buffer could prevent the binding of nucleic acid to a solid phase and thus, a step was required to remove or neutralize the effect of said surfactant. This was reflected in step (b) of claim 1. In the context of the claim, step (b) was an active step that implicitly required neutralizing the effect of the cationic surfactant present in the disrupting buffer so as to permit the binding of nucleic acid to a solid phase, as explicitly stated.

In paragraphs [006] and [007], under the heading "Summary of the invention", methods and kits of the invention were described in general terms. The methods were further described in paragraph [008], wherein a disrupting buffer and the substantial neutralization of a cationic surfactant present in this buffer (the feature "**substantially neutralizing**") were explicitly

mentioned; this corresponded to the method defined in claim 1 of the patent application. Paragraphs [009] and [010] described two kits, one of them defined as comprising a non-ionic surfactant characterized as permitting the binding of nucleic acid to a solid phase in the presence of the disrupting buffer and a high salt buffer (the feature "**permits binding**"); this part of the description corresponded to the kit of claim 64 of the patent application. It was thus directly derivable from this section alone that both kits, in particular the kit of claim 64, could be used in the methods previously described, in particular the method of claim 1 of the patent application.

Furthermore, paragraph [062], under the heading "Detailed description of the embodiments", described the methods of the invention, defined the feature "substantially neutralizing", and referred in general terms to possible methods for accomplishing this neutralization. In paragraph [063], the addition of a reagent, such as a second (non-ionic) surfactant, was described as a possible alternative. The methods of the invention were further described in paragraphs [086] and [087] under the heading "Exemplary Embodiments". Paragraph [087] described the method of claim 1 comprising the feature "substantially neutralizing". Two alternatives were presented to achieve neutralization. The first contemplated the removal of the cationic surfactant (paragraph [089]), the second the addition of a second surfactant (paragraph [090]), as in the method of claim 1 of the patent application. Paragraph [0105] described a kit containing a second (non-ionic) surfactant and a buffer with a high salt concentration, like the kit of claim 64. Paragraph [0106] provided the reason for having a buffer with a high salt concentration, namely to permit the binding

of the nucleic acid to a solid phase (the feature "permits binding"). The use of a (second) non-ionic surfactant and a buffer with a high salt concentration permitted the binding of more nucleic acid to a solid phase, than in the absence of said compounds. It thus had a quantitative effect. The combination of features from these paragraphs resulted, directly and unambiguously, in the method of claim 1 of auxiliary request 1.

According to paragraph [071], high salt concentrations were required for nucleic acids to bind to a solid phase, i.e. it was necessary to have a buffer with a high salt concentration before carrying out step (c) of claim 1. However, paragraph [0106] described that, for some cationic surfactants, the presence of high salt concentrations could prevent such a binding. As described *inter alia* in paragraphs [068] and [090], this problem could be solved by adding a second surfactant, in particular a non-ionic surfactant. The two embodiments falling within step (b) of claim 1 of auxiliary request 1, namely a stepwise addition or a simultaneous addition of a non-ionic surfactant and a buffer with a high salt concentration, were directly derivable from these disclosures. The binding solutions described in Examples 21 and 22 of the patent application supported this conclusion and exemplified one of these embodiments.

XII. The submissions made by the respondent, insofar as relevant to the present decision, may be summarised as follows:

Admission of the main request

The relevance of the comma in step (b) of claim 1 had been an essential issue in opposition proceedings. It was already raised in the opponent's submissions filed before oral proceedings at first instance, wherein the differences in step (b) of granted claim 1 (without a comma), claim 1 of the "Druckexemplar" (with a comma) and claim 1 of the auxiliary requests then on file (with a semicolon), were discussed in detail. The issue was also discussed at length during the oral proceedings at first instance. In view thereof, the absence of a comma in step (b) of claim 1 of all requests filed with the grounds of appeal was not an error but a deliberate amendment of claim 1.

Although the respondent, in its response to the statement of grounds of appeal, had referred to the absence of the comma in step (b) of claim 1 of all claim requests, the appellant had deliberately waited for the board's provisional opinion and, only thereafter, filed requests to address the alleged error. If the omission of the comma had indeed been an error, the appellant could have corrected it by immediately filing new requests instead of waiting more than four years. The appellant's behaviour was not in line with the case law that required a party to act with due diligence and, for an appellant, to present its complete case in the statement of grounds of appeal.

Admission of the auxiliary request 1

As for the main request, auxiliary request 1 was not admissible due to the introduction of a comma in step (b) of claim 1. The differences between claim 22 of auxiliary request 1 in appeal and at first instance did not change the technical meaning of the claim.

Auxiliary request 1
Article 123(2) EPC

The combination of claims 1, 4 and 9 of the patent application resulted in a method using a (second) non-ionic surfactant (claim 9) characterized by the feature "substantially neutralizing", not by the feature "permits the binding". The combination of claims 1, 4 and 9 did not thus provide a basis for the method of claim 1 of auxiliary request 1.

Whilst the feature "substantially neutralizing" required an active step (neutralization of the effect of the cationic surfactant), the feature "permits binding" did not require any active step but only to permit the binding of nucleic acid to a solid phase. Both features had different technical meanings and were not interchangeable. Paragraph [008] of the patent application referred to two methods but the feature "substantially neutralizing" was mentioned in only one of them. Likewise, the feature "substantially neutralizing" was present only in the method of claim 1 but not in the method of claim 25 of the patent application.

Paragraph [062] of the patent application defined "substantially neutralizing" as a quantitative, measurable feature requiring that, as a result of the neutralization, more nucleic acid was bound to a solid phase than without neutralization. This quantitative requirement was not reflected in the feature "permits binding" which was not defined in the patent application and disclosed only in conjunction with one of the two kits described in the patent application (the kit of claim 64). All of the methods described in

the patent application, such as, *inter alia*, in paragraphs [087] and [090] referred only to "substantially neutralizing".

Whilst the kit of claim 41 comprised a second surfactant characterized as "substantially neutralizing" the cationic surfactant, the kit of claim 64 comprised a non-ionic surfactant characterized as "permitting the binding of nucleic acid". A kit was characterized by the compounds comprised in it but its composition did not provide any information on how to use them, i.e. when to apply these compounds (one-step, stepwise) and under which conditions (concentration, type of solid phase). The patent application did not describe how to use the kit of claim 64 or in which of the disclosed methods it could be used. The kit of claim 64 could thus not serve as an implicit basis for the method of claim 1 of auxiliary request 1.

Paragraph [0105] of the patent application described the kit of claim 64, and paragraph [0106] the effect of a high salt concentration on the cationic surfactants but without a reference to a second, non-ionic surfactant. The manner in which a non-ionic surfactant and a buffer with high salt concentration were added to the reaction composition was also not derivable from paragraphs [068], [071] and [0106].

Examples 21 and 22 of the patent application only described the simultaneous addition of a non-ionic surfactant and a buffer with high salt concentration (binding solution). There was no disclosure in the patent application supporting a stepwise addition of these two compounds. This embodiment represented an intermediate generalisation with no basis in the patent application.

XIII. The appellant (patent proprietor) requested to set aside the decision under appeal and to maintain the patent on the basis of the main request or, alternatively, upon the basis of one of the auxiliary requests 1 to 13, all filed under cover of a letter dated 13 January 2018.

XIV. The respondent (opponent) requested to dismiss the appeal.

Reasons for the Decision

Main request
Admission into the appeal proceedings

1. The main request has been filed in reply to the board's communication and after oral proceedings had been arranged (Article 13(3) RPBA). It represents thus an amendment to the appellant's case and may be admitted at the board's discretion (Article 13(1) RPBA). When exercising its discretion the board considers, *inter alia*, the complexity of the new subject-matter, the current state of the proceedings and the need for procedural economy.

Claim 1

2. In the communication pursuant to Article 15(1) RPBA, the board referred to the function of an appeal as established by the case law of the Boards of Appeal (cf. "Case Law of the Boards of Appeal of the EPO", 8th edition 2016, IV.E.1, 1065) and informed the parties that it was minded not to admit any of the appellant's claim requests filed with the statement of grounds of appeal. The board arrived at this provisional opinion

because, *inter alia*, a comma which had been present in step (b) of claim 1 of all requests underlying the decision under appeal, was missing in step (b) of all requests filed with the grounds of appeal.

3. The main request is identical to the main request filed with the statement of the grounds of appeal except for the re-introduction of the comma in step (b). The amendment is thus a direct reaction to the comments in the board's communication which does not raise any new issues and simplifies the appeal proceedings. The subject-matter of claim 1 of the main request was examined by the opposition and a decision taken thereupon (c. pages 7 to 11, points 3 to 5 of the decision under appeal).

Dependent claims 23 to 25

4. In the communication pursuant to Article 15(1) RPBA, the board drew the parties' attention to the case law on the filing of requests that had been filed and subsequently withdrawn in proceedings at first instance (Article 12(4) RPBA; cf. *inter alia*, T 361/08 of 3 December 2009, point 13 of the Reasons, and T 679/09 of 13 November 2012, point 12 of the Reasons). The board noted that deficiencies under Article 84 EPC of claims directed to a kit had already been dealt with during the opposition procedure and amended claim requests had been filed to address them (cf. points 2.2 and 2.3 of the "Summons to attend oral proceedings" issued by the opposition division; and claims 22 and 23 of auxiliary requests 1 and 2 filed by the patent proprietor on 15 March 2013 in reply thereto). None of these auxiliary requests were prosecuted at the oral proceedings before the opposition division because the patent proprietor replaced all requests then on file by

a new main request and new auxiliary requests 1 to 3; the requests underlying the decision under appeal.

5. Claim 22 of the main request differs from claim 22 of the main request underlying the decision under appeal by the deletion of subject-matter defining the chemical nature of the ribonuclease inhibitor (cf. point IX *supra*). The deletion of this subject-matter in claim 22 and its introduction in dependent claims 23 to 25 attempts to overcome the objection raised under Article 84 EPC against the main request underlying the decision under appeal (cf. pages 10 and 11, point 5 of the decision under appeal).

6. The scope of claim 22 of the main request is identical to that of claim 22 of auxiliary request 1 of the decision under appeal. However, the main request additionally comprises dependent claims encompassing subject-matter which, as such, was not examined and decided upon by the opposition division. The subject-matter of dependent claims 23 to 25 was not present in any of the auxiliary requests underlying the decision under appeal. The introduction of this subject-matter into the appeal proceedings is not in line with the case law referred to above.

Conclusion

7. Thus, the board, exercising its discretion pursuant to Article 13(1) RPBA, does not admit the main request into the appeal proceedings.

Auxiliary request 1

Admission into the appeal proceedings

8. The introduction of a comma in step (b) of claim 1 of auxiliary request 1 is considered to be allowable (cf. points 2 and 3 *supra*).

9. Claim 22 of auxiliary request 1 is not identical to claim 22 of auxiliary request 1 at first instance but the differences are minor amendments of an editorial nature which do not change, as acknowledged by the respondent, the scope and technical meaning of the claim. The subject-matter of this claim was thus examined and decided upon at first instance.

10. Therefore, the board, exercising its discretion pursuant to Article 13(1) RPBA, admits auxiliary request 1 into the appeal proceedings.

Auxiliary request 1
Interpretation of claim 1

11. In the communication pursuant to Article 15(1) RPBA, the board referred to the established case law on the interpretation of claims and drew the parties' attention to several issues that it considered relevant (cf. point 15 of the board's communication).

11.1 The term "comprising" in the preamble of claim 1 does not exclude the presence of additional (for instance intermediate) steps as far as these steps do not contravene the purpose of the claimed method (cf. "Case Law", *supra*, II.A.6.2, 288; *inter alia*, T 457/02 of 16 November 2005, points 4.2 and 4.3 of the Reasons).

11.2 The sequence of steps in a method claim informs a skilled person not only that the method comprises several steps but also about the order in which they are carried out. Step (b) of claim 1 is thus a

different, separate step from step (a) (two-step interpretation). This interpretation excludes embodiments in which steps (a) and (b) are carried out simultaneously (open interpretation).

11.3 Step (a) of claim 1 requires to contact the biological sample with a disrupting buffer comprising a protease, a cationic surfactant and an agent providing for said buffering effect. Step (a) requires thus the provision of a single solution or composition. Thereby, step (a) excludes embodiments that comprise the separate or stepwise addition of a cationic surfactant, a protease and a buffering agent.

11.4 Step (b) of claim 1 requires the addition of a second non-ionic surfactant and a buffer with high salt concentration. The wording of step (b) allows two possible interpretations, namely the simultaneous addition of a second non-ionic surfactant and a buffer with high salt concentration, and the stepwise addition of a second non-ionic surfactant and, separately, a buffer with high salt concentration. Both embodiments are within the scope of the claim.

11.5 The feature **"permits binding"** in step (b) of claim 1 is a functional feature defining the properties of the second non-ionic surfactant and it has been given two different interpretations.

In a first interpretation (cf. points 4.3 and 4.3.1 of the decision under appeal), the feature "permits binding" is equated to the feature **"substantially neutralizing"**, which is defined in the patent application as meaning that "more nucleic acid in a sample is capable of binding a solid phase with such substantial neutralization than without the

neutralization" (cf. paragraph [0062] of the patent application). According to this narrow interpretation, the feature "permits binding" is thus comparative and has a quantitative character. In a second broader interpretation, the feature "permits binding" merely requires the second non-ionic surfactant not to block, prevent or inhibit the binding of nucleic acid to a solid phase in the presence of the protease, the cationic surfactant and the buffering agent (the disrupting buffer) of step (a) of claim 1, i.e. the second non-ionic surfactant must permit the binding of nucleic acid to a solid phase in the presence of the disruptive buffer but it does not need to substantially neutralize the (effect of the) cationic surfactant present in the disrupting buffer.

The case law of the boards states that, if the features of a claim are clear in themselves, the board can restrict its assessment of the meaning of these features to the wording and structure of the claim, and reference to the description is not required (cf. "Case Law", *supra*, I.C.4.8, 110; *inter alia*, T 2487/12 of 27 October 2015, point 1.13 of the Reasons). In the light of this case law the board sees no reason for equating the features "permits binding" and "substantially neutralizing", excluding thereby a broader, equally possible interpretation of the former feature. In the board's view, this broader interpretation is not illogical and makes technical sense (cf. "Case Law", *supra*, II.A.6.1, 287; *inter alia*, T 190/99 of 6 March 2001, point 2.4 of the Reasons).

Article 123(2) EPC

12. The respondent argues that claim 1 of auxiliary request 1, due to the presence of the feature "permits binding" in step (b), contravenes Article 123(2) EPC.

13. Throughout the whole proceedings at first instance and in the parties' submissions in appeal proceedings, it has not been contested that all methods disclosed in the patent application contain the feature "substantially neutralizing" and that there is no explicit disclosure in the patent application of a method containing the feature "permits binding" (cf. paragraphs [008], [062] and [087] of the patent application). This is also reflected in the claims. The method of claim 1 of the patent application comprises a disrupting buffer and explicitly refers to "substantially neutralizing the cationic surfactant". This neutralization is further defined in claim 4 as "adding a second surfactant that substantially neutralizes the cationic surfactant" and, in claim 9, the second surfactant is defined as "a non-ionic surfactant". Claim 6 requires "adding a salt" but without any reference to its concentration. Since the features "permits binding" and "substantially neutralizing" are not interchangeable (cf. point 11.5 *supra*), none of these disclosures provides a basis for the subject-matter of claim 1 of auxiliary request 1.

14. Subject-matter not explicitly disclosed can nevertheless be implicitly disclosed in a patent application. The criteria for an implicit disclosure have been defined in the case law. In particular, the Boards consider that an implicit disclosure must derive directly and unambiguously from the content or teaching conveyed by the original disclosure, i.e. it must be a clear and unambiguous consequence of what is explicitly mentioned in the patent application; it is not however

subject-matter that may be rendered obvious on the basis of the content of the patent application (cf. "Case Law", *supra*, II.E.1.2.2, 405, and 1.2.3.a), 407; *inter alia*, T 389/13 of 19 September 2017, points 3.1 to 3.4 of the Reasons, and T 782/16 of 18 July 2017, point 4.1.3 of the Reasons). It remains thus for the board to assess whether the content of the patent application provides such an implicit disclosure for the method of claim 1 of auxiliary request 1.

15. It has not been contested that the feature "permits binding" is only found in association with one of the two kits disclosed in the patent application. Whilst the kit of claim 41 comprises a second surfactant that "substantially neutralizes the cationic surfactant" (emphasis by the board; cf. paragraph [096] of the patent application), the kit of claim 64 comprises a non-ionic surfactant that "permits the binding of nucleic acid to a solid phase in the presence of the protease and cationic surfactant; and a buffer with a high salt concentration" (emphasis by the board; cf. paragraph [0105] of the patent application). The characterization of the non-ionic surfactant comprised in the product (kit) of claim 64 is identical to the characterization of the non-ionic surfactant used in the method of claim 1 of auxiliary request 1 and, indeed, this kit has been given as an implicit basis for the latter.

16. However, although the compounds comprised in the kit of claim 64 are identical to those used in the steps of the method of claim 1, including a buffer with a high salt concentration and a non-ionic surfactant characterized by the feature "permits binding", the composition of the kit as such does not provide any information on how these compounds are used. Should

they be added simultaneously or stepwise, and, if stepwise, as sub-combinations or individually and in which (sequential) order? The addition of a compound in the presence or absence of other compounds (previously added or added latter on), the specific conditions used in one step of the method, etc. may all lead to very different outcomes. Nevertheless, it may be reasonable to assume that, since the kit is disclosed in the patent application, it may well be intended - and thus, be appropriate and suitable - for use in at least one or some of the methods described therein.

17. Several methods are disclosed in the patent application and described at different levels of generalization. Whilst the most generic disclosures are at the beginning of the description, the most specific are disclosed in the examples of the patent application. In the generic disclosures, there is no indication of the steps carried out, if any (cf. paragraphs [006] and [008] of the patent application).

17.1 Paragraph [062], under the heading "Detailed description of the embodiments", refers to certain embodiments in which the methods rely on "substantially neutralizing" a cationic surfactant and this neutralization "is accomplished by the conditions in the disrupting buffer, and do not necessarily comprise a separate step from contacting the biological sample with the disrupting buffer" (single, one-step method; cf. page 21, lines 1 to 3). Immediately thereafter in paragraph [063], other embodiments are described in which the neutralization "may include, but is not limited to, ... precipitating the cationic surfactant, removing the surfactant by phase extraction, ... by dialysis ... [or] by other means" (stepwise methods; emphasis by the board). Paragraph [063] also refers to

other embodiments in which "reagents which substantially neutralize the cationic surfactant include, but are not limited to, chaotropes, nonionic surfactants, anionic surfactants, and zwitterionic surfactants" (emphasis by the board). However, nothing is said about how any of these reagents are added to the biological sample or reaction composition.

17.2 Similar disclosures are found under the heading "Exemplary embodiments". Paragraph [068] states that "[i]n certain embodiments, these cationic surfactant:nucleic acid complexes may be dissolved using a nonionic surfactant and an appropriate salt". However, reference is also made to other embodiments "using a zwitterionic or anionic surfactant, and an appropriate salt" and, in more general terms, to a "second surfactant and a salt". Indeed, if nucleic acid is isolated using a solid phase, the absorption or binding of nucleic acid to the solid phase is carried out "in the presence of high concentrations of a chaotrope or salt" if the support is derived from silica (silica particles, silicon dioxide, etc.), or "in the presence of low ionic strength" if the support is an ion exchange resin (Chromex, DEAE Sepharose, etc). Whilst for silica supports, the nucleic acid is "eluted from the solid phase using a solution with a low ionic strength", for the ion exchange resin, the elution is performed "by increasing the ionic strength" (cf. paragraphs [071] and [072] of the patent application). Other solid phase materials are also explicitly mentioned (cf. page 24, last line to page 25, third line).

17.3 Paragraph [086] refers again in general terms to methods for obtaining nucleic acid from a biological sample and binding the nucleic acid to a solid phase.

Paragraph [087] describes further methods comprising a step of "substantially neutralizing the cationic surfactant". Whilst in the methods described in paragraph [089] "substantially neutralizing of the cationic surfactant is accomplished by substantially removing the cationic surfactant" and "such methods may include, but are not limited to, one or more of precipitation, phase extraction, and dialysis" (emphasis by the board), in the methods described in paragraph [090] "substantially neutralizing of the cationic surfactant comprises adding a second surfactant that substantially neutralizes the cationic surfactant", wherein in certain embodiments, "the second surfactant is a nonionic surfactant".

17.4 A first kit, namely the kit of claims 41 to 63 of the patent application, is described in paragraphs [096] to [0104], and a second, corresponding to the kit of claim 64, is described in paragraph [0105]. The subsequent paragraph [0106] refers to "the process of combining the process of releasing nucleic acid from samples using cationic surfactants with the process of binding the nucleic acid to a solid phase into a single nucleic acid isolation process" (emphasis by the board), and to the effect of "the high salt concentrations" on certain cationic surfactants and on the binding of nucleic acids to a solid support.

17.5 Examples 21 and 22 disclose a method for obtaining nucleic acid from a biological sample, where in a first step a "disrupting buffer" comprising a protease (Proteinase K) and a cationic surfactant (DTAB) and a low salt concentration (20 mM $CaCl_2$) is used, and in a second step a "binding solution" comprising a non-ionic surfactant (Tween 20) in a high salt concentration

(5M GuSCN) is added, before binding the nucleic acid to a solid phase (cf. paragraphs [0179] to [0187] of the patent application). Examples 4, 17 and, in particular, Example 20 show that the non-ionic surfactant used in Examples 21 and 22 "substantially neutralizes the cationic surfactant". This is also reflected in the combinations of the (method) claims 1, 4, 9 and 11 to 13 and of the (product) claims 41, 59 and 61 to 63 of the patent application.

18. In view of this disclosure, the board considers that, for some of the disclosed methods, the use of a kit according to claim 64 may be appropriate. These methods may not necessarily require the use of a non-ionic surfactant "substantially neutralizing the cationic surfactant". It may be sufficient that the non-ionic surfactant does not interfere with, i.e. permits, the binding of the nucleic acid to a solid phase in the presence of a protease and a cationic surfactant.

19. In the present case, the decisive question is whether, in view of all the methods explicitly mentioned, the patent application directly and unambiguously, yet in an implicit manner, discloses a method as defined in claim 1 of auxiliary request 1. In particular, since the method described in Examples 21 and 22 of the patent application is the most similar to that of claim 1, the question arises whether the patent application directly and unambiguously discloses the use of the kit of claim 64 in the method described in these examples.

20. In the board's view, the use of the kit of claim 64 for performing this method - or the substitution of the non-ionic surfactant used in Examples 21 and 22 by a non-ionic surfactant that "permits the binding of the

nucleic acid to a solid phase" but does not "substantially neutralize the cationic surfactant" - is not directly and unambiguously disclosed in the patent application, let alone the use of the kit for performing the more generally defined method of claim 1 of auxiliary request 1.

21. The method of claim 1 of auxiliary request 1 is thus not directly and unambiguously derivable from the content of the patent application, not even in an implicit manner.

22. The board further observes that the methods described in Examples 21 and 22 of the patent application are based on the provision of two particular compositions, namely a "disrupting buffer" and a "binding solution", the latter comprising a non-ionic surfactant and a buffer with a high salt concentration. However, step (b) of claim 1 of auxiliary request 1 embraces not only this embodiment, comprising the use of a single binding solution, but a second embodiment in which the non-ionic surfactant is first added to the reaction composition and, only afterwards, the buffer with a high salt concentration is added. There is however no basis for this second embodiment in the patent application; the addition of a buffer with high salt concentration is always disclosed in a generic manner (in the description and claims of the patent application) or in the specific disclosure of Examples 21 and 22.

23. To conclude, claim 1 of auxiliary request 1 contravenes Article 123(2) EPC.

Auxiliary requests 2 to 13

24. Step (b) of claim 1 of auxiliary requests 2 to 13 comprises the same feature "permits binding" as claim 1 of auxiliary request 1. In view of the board's conclusion on this feature in auxiliary request 1, auxiliary requests 2 to 13 are in any case unallowable.

25. In the absence of an allowable request, the appeal has to be dismissed.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated