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Datasheet for the decision of 3 September 2021

Case Number: T 0366/16 - 3.3.08

Application Number: 09784897.2

Publication Number: 2329019

IPC: C12N15/10

Language of the proceedings: EN

Title of invention:
Isolation of nucleic acid

Patent Proprietor:
Cambridge Enterprise Limited

Opponent:
Qiagen GmbH

Headword:
Method and kit for nucleic acid isolation/CAMBRIDGE

Relevant legal provisions:
EPC Art. 56
RPBA 2020 Art. 13(2)

Keyword:
Auxiliary requests 6 to 8 - inventive step - (no)
Auxiliary requests 9 to 11 - Admission into the proceedings - (no)

Decisions cited:
T 1852/11

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 0366/16 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 3 September 2021

Appellant 01: (Patent Proprietor)	Cambridge Enterprise Limited The Old Schools Trinity Lane Cambridge Cambridgeshire CB2 1TN (GB)
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Appellant 02: (Opponent)	Qiagen GmbH Qiagen Str. 1 40724 Hilden (DE)
Representative:	Roth, Carla König-Szynka-Tilmann-von Renesse Patentanwälte Partnerschaft mbB Mönchenwerther Straße 11 40545 Düsseldorf (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 7 December 2015 concerning maintenance of the European Patent No. 2329019 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. Montrone
	F. Bostedt

Summary of Facts and Submissions

I. The appeal lies against the decision of an opposition division to maintain the European patent No. 2 329 019 in amended form. The patent was filed under the PCT and published as international patent application WO 2010/015835 (hereinafter the "patent application").

II. The opposition division considered the main request to contravene Article 123(2) EPC. Since auxiliary requests 1 and 2 suffered *prima facie* from the same deficiency, they were not admitted into the proceedings, while all late filed documents, including documents D13 to D16, were admitted. Auxiliary request 3 was held to fulfil the requirements of the EPC.

III. With their statement of grounds of appeal, the patent proprietor (hereinafter "appellant I") submitted a main request and auxiliary requests 1 to 3, which were all identical to those dealt with in the decision under appeal.

IV. With their statement of grounds of appeal, the opponent (hereinafter "appellant II") submitted that the subject-matter of claims 1, 14 and 21 of auxiliary request 3 as maintained by the opposition division lacked novelty over document D2, or lacked an inventive step starting *inter alia* from document D2 as closest prior art.

V. In reply to appellant II's statement of grounds of appeal, appellant I submitted counter-arguments in support of novelty and inventive step of auxiliary request 3. Appellant I further submitted auxiliary requests 4 and 5, which were new to the proceedings.

VI. Appellant II replied to appellant I's statement of grounds of appeal.

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

VIII. In reply, appellant I submitted auxiliary requests 6 to 8.

IX. Oral proceedings before the board were held on 3 September 2021 by video conference as requested by the parties. During the oral proceedings, appellant I submitted auxiliary requests 9 to 11, and withdrew the main request, as well as auxiliary requests 1 to 5.

X. Claims 1 and 13 of auxiliary request 6 read:

"1. A method for isolating a nucleic acid, which comprises:

(i) binding the nucleic acid to a solid phase at a first pH in the presence of a binding buffer, wherein the first pH is an acidic pH;

(ii) washing the bound nucleic acid with a wash solution; and

(iii) eluting the nucleic acid from the solid phase at a second pH which is higher than the first pH, wherein the second pH is in the range pH 6.5-10;

wherein the wash solution comprises a buffer with a buffering range that encompasses a pH that is higher than the first pH, and the wash solution is at a pH

that is within a buffering range of the binding buffer but lower than the buffering range of the buffer of the wash solution, and wherein the pH of the wash solution is pH 6.0 or less; wherein the method is carried out in the absence of a chaotropic agent and in the absence of an organic solvent.

13. A kit for isolation of a nucleic acid, which comprises:

i) a binding buffer for binding the nucleic acid to a solid phase at a first pH, wherein the first pH is an acidic pH; and

ii) a) a wash solution that comprises a buffer with a buffering range that encompasses a pH that is higher than the first pH, wherein the wash solution is at a pH that is within a buffering range of the binding buffer but lower than the buffering range of the wash buffer, and wherein the pH of the wash solution is pH 6.0 or less; and optionally

b) a solution for eluting the nucleic acid from the solid phase, wherein the solution is at a second pH that is higher than the first pH, and wherein the second pH is in the range pH 6.5 - 10; or

iii) a) a composition in dry form that when dissolved in a liquid provides a wash solution according to (ii) (a); and optionally

b) a composition in dry form that when dissolved in a liquid provides a solution according to (ii) (b)

wherein the kit does not comprise a chaotropic agent, and the kit does not comprise an organic solvent."

XI. Claims 1 and 13 of auxiliary request 7 differ from the respective claims of auxiliary request 6 in that the feature "*wherein the second pH is within the buffering range of the wash buffer*" has been added.

XII. Claims 1 and 13 of auxiliary request 8 differ from the respective claims of auxiliary request 6 in that the feature "*wherein the second pH is within the buffering range of the wash buffer*" has been added, and in that the feature "*pH 6.5-10*" has been replaced by "*pH 7-9*".

XIII. Claim 1 of auxiliary request 9 differs from claim 1 of auxiliary request 6 in that the features "*wherein the nucleic acid is eluted from the solid phase in the presence of an elution buffer, wherein the second pH is within a buffering range of the elution buffer*" has been added. Moreover, all claims relating to a kit have been deleted.

XIV. Claim 1 of auxiliary request 10 differs from claim 1 of auxiliary request 6 in that the features "*wherein the second pH is within the buffering range of the wash buffer*" and "*wherein the nucleic acid is eluted from the solid phase in the presence of an elution buffer, wherein the second pH is within a buffering range of the elution buffer*" have been added. Moreover, all claims relating to a kit have been deleted.

XV. Claim 1 of auxiliary request 11 differs from claim 1 of auxiliary request 6 in that the features "*wherein the second pH is within the buffering range of the wash buffer*" and "*wherein the nucleic acid is eluted from*

the solid phase in the presence of an elution buffer, wherein the second pH is within a buffering range of the elution buffer" have been added, and in that the feature "*pH 6.5-10*" has been replaced by "*pH 7-9*". Moreover, all claims relating to a kit have been deleted.

XVI. The following documents are cited in this decision:

D2: US 6,355,792 (published on 12 March 2002);

D3: WO 00/29562 (published on 25 May 2000);

D13: mirVana™ miRNA Isolation, Ambion, Catalog #1560, Version 0401 (January 2004);

D14: RNeasy™, Mini Handbook, Qiagen, 2001, 1-11;

D15: QIAquick™, Spin Handbook, Qiagen, 2002, 1-18;

D16: RiboPure™-WBC, Instruction Manual, Ambion, Catalog #1903, Version 0208, published on 17 April 2003.

XVII. Appellant I's submissions, insofar as relevant to the present decision, may be summarised as follows:

Admission of documents D13 to D16 into the appeal proceedings

The opposition division admitted the late filed documents D13 to D16 into the opposition proceedings. Although these documents were filed by appellant II (then the opponent) in response to appellant I's (then the patent proprietor) new sets of claims and arguments, they lacked *prima facie* relevance. The opposition thus erred in admitting them.

Auxiliary request 6

Novelty

The method of claim 1 was not anticipated by the disclosure of document D2. Example 3 of this document disclosed in conjunction with the Table in Example 1, that the Tris-HCl wash buffer was used at pH 6.5. Although column 3, lines 36 to 39 of document D2 disclosed generally that phosphate-free wash buffers were used preferably at a pH "*between 4.5 and 6.5*", the binding buffers, for example in Example 3, could be used for washing too. Since, moreover, there was no teaching that every phosphate-free wash buffer should be used over the whole of the preferred pH range but different ones at individual pH values, and Tris-HCl was not the sole available phosphate-free wash buffer, document D2 did not directly and unambiguously disclose the use of Tris-HCl as a wash buffer at pH 6 or lower.

Inventive step

The MES binding buffer embodiment of Example 3 in document D2 represented the closest prior art. This embodiment disclosed that for the isolation of nucleic acids the carrier material was washed with a Tris-HCl buffer at pH 6.5, while in claim 1 the wash buffer was used at pH 6.0 or lower.

This difference allowed the use of different binding buffers, different wash buffers and different combinations of binding and wash buffers, whilst still obtaining a high yield in the absence of a chaotropic agent and an organic solvent.

The technical problem was hence the provision of an improved method for nucleic acid isolation.

Although no comparative example was available with the MES binding buffer embodiment of document D2, the EPC did not require such a comparison. It sufficed that the improvement was credible over the whole range claimed in light of the data provided in the patent.

Examples 1, 3 and 4 of the patent disclosed that this improvement was achieved across the whole breadth of claim 1, since a high yield was obtained with a combination of various binding buffers and wash buffers at different pH values and different elution conditions. Furthermore, the description of the patent disclosed the use of various carrier materials, including a so called "charge-switch" material, which rendered it plausible that the invention could be carried out across the whole breadth claimed (see paragraphs [0016] and [0019]). There was also no need that the two conditions set out in paragraph [0049] of the patent were fulfilled to achieve a high yield with the claimed method. Example 3 of the patent demonstrated that a high yield was obtained even if the second pH used for eluting the nucleic acids was not within the buffering range of the wash buffer. It sufficed that the wash buffer was used at an acidic pH that was lower than its buffering range. Example 4 disclosed that, in comparison to a commercial kit, the yield was at least comparable without using chaotropic salts and organic solvents.

Contrary to the patent, document D2 disclosed that solely a single combination of binding buffer and wash buffer achieved a high yield of isolated nucleic acids. Table 3 of document D2 disclosed a yield of 70% for a

MES buffer used at pH 5.5 for binding the nucleic acids to the carrier material if combined with a Tris-HCl wash buffer at pH 6.5. However, a yield of 90% was solely achieved by using an acetate buffer at pH 4.5 for binding combined with a Tris-HCl buffer at pH 6.5.

As regards obviousness, document D2 taught that the binding pH and the choice of binding buffer were the essential factors for achieving a high yield. Furthermore, the highest yield was obtained with a single combination of binding buffer and wash buffer at defined pHs. The skilled person in view thereof had no motivation to change these conditions with any reasonable expectation of success. The wash buffer's use was indicated as optional only, highlighting its low importance. Document D2 further lacked pointers to an improvement of the yield mediated by lowering the Tris-HCl wash buffer's pH of 6.5 to 6.0. Rather on the contrary, such a low pH was further removed from Tris-HCl's buffering range (pH 7.1 to 9). If the skilled person had nevertheless changed the optimised conditions as disclosed in Example 3 of document D2, then instead of lowering Tris-HCl's pH for washing, a binding buffer had been used for washing (see column 3, line 37 of document D2). The binding buffers in Example 3 were used in the pH range of 4.5 to 6.5 (see Table in column 6 of document D2), which was identical to the indicated preferred pH range for washing (see column 3, line 39).

Admission of auxiliary requests 9 to 11 filed at the oral proceedings into the appeal proceedings

Auxiliary requests 9 to 11 were filed to address the board's new argument that it was not credible that when water was used for eluting the nucleic acids from the

carrier material in combination with acidic binding buffers and wash buffers, the claimed method did not achieve a high yield across the whole breadth of claim 1. This argument was only raised for the first time during the oral proceedings. These exceptional circumstances justified the admission of auxiliary requests 9 to 11 at this late stage of the proceedings.

XVIII. Appellant II's submissions, insofar as relevant to the present decision, may be summarised as follows:

Admission of documents D13 to D16 into the appeal proceedings

Although documents D13 to D16 were filed three days prior to oral proceedings, they represented an immediate response to the new set of claims and arguments submitted by appellant I only eight days before the oral proceedings. These documents were filed in support of an inventive step argument submitted earlier. It was within the discretionary power of the opposition division to admit these documents into the proceedings. This was done in a reasonable and correct manner.

Auxiliary request 6

Novelty

The method of claim 1 lacked novelty over the disclosure of document D2. It was uncontested that the MES binding buffer embodiment of Example 3 in document D2 disclosed all the features of the claimed method, except for the use of a wash buffer at pH 6 or lower. Example 3 in conjunction with the Table in Example 1 of document D2 disclosed a study that assessed the yield

of isolated nucleic acids using silica particles in the presence of various binding buffers at different pHs. The bound nucleic acids in Example 3 were washed with a Tris-HCl buffer at pH 6.5. This buffer was the sole exemplified wash buffer disclosed in document D2. Since, moreover, column 3, lines 36 to 39 of document D2 disclosed that wash buffers were preferably used in the pH range of 4.5 to 6.5, the skilled person at least seriously contemplated the use of Tris-HCl at a lower pH than 6.5 too, i.e. in the whole preferred pH range of 4.5 to 6.5. There were also no reasons apparent that the skilled person was prevented from doing so, and Tris-HCl's use as wash buffer in the examples of document D2 was in line with the document's overall teaching.

Inventive step

Document D2 represented the closest prior art for the method of claim 1. This document disclosed a method for the isolation of nucleic acids from liquids based on a solid carrier material using acidic conditions between pH 1 to 6 for binding purposes. These conditions resulted in rapid binding of the nucleic acids and allowed their subsequent elution from the carrier material under alkaline conditions, i.e. by a simple pH switch. A preferred washing step was mentioned (see column 1, lines 4 to 6, and column 2, lines 5 to 10, 31 and 32). The washing occurred at acidic conditions too, preferably in the pH range of 4.5 to 6.5 to avoid any premature elution of the bound nucleic acids (see column 3, lines 36 to 48). Tris-HCl was used on purpose as wash buffer. Its use allowed the subsequent elution of the nucleic acids under alkaline conditions by a simple pH switch from pH 6.5 to 9 without changing the buffer, due to Tris-HCl's alkaline buffering range (pH

7.1 to 9). The use of Tris-HCl for elution purposes was thus indicated as preferred (see Examples 1 to 3, and column 3, lines 59 to 65). Example 3 further disclosed that the binding of nucleic acids to solid carriers at lower pHs resulted in higher yields (see Table in column 6).

The generic concept derivable from document D2 was therefore that the binding and washing of nucleic acids occurred at an acidic pH, while for the elution alkaline conditions were required. Tris-HCl was the preferred buffer for washing and eluting nucleic acids.

The MES binding buffer embodiment in Example 3 of document D2 was the closest prior art for the claimed method. It differed from claim 1 solely in the use of a wash buffer at pH 6.5 instead of pH 6.0, i.e. by a 0.5 pH difference. There was no evidence available that this small pH difference was associated with an advantageous technical effect. None of the working examples in the patent disclosed an absolute yield of the nucleic acids obtained, i.e. the yield disclosed was relative, while document D2 disclosed an absolute yield. Accordingly, the Examples in the patent allowed no conclusions to be drawn about the efficacy of the claimed method in isolating nucleic acids compared to the MES binding buffer embodiment of document D2, let alone for the whole range claimed. Document D2 likewise did not use a chaotropic salt or any organic solvents for isolating nucleic acids. A comparison of the claimed method with an undefined commercial product using these hazardous agents as shown in Example 4 of the patent was therefore irrelevant.

In the absence of any improvements of the claimed method compared to the closest prior art method the

technical problem was the provision of an alternative nucleic acid isolation method.

The use of the Tris-HCl buffer at pH 6 or lower was obvious, since document D2 taught that the preferred range for wash buffers was in between pH 4.5 and 6.5 (see column 3, line 39). In looking for alternative washing conditions, the skilled person would have used the wash buffer within the complete preferred pH range, in particular, since reasons for not using Tris-HCl in this range were not apparent. The use of Tris-HCl as wash buffer at a pH lower than 6.5 was likewise known from the prior art (see e.g. document D3, page 4, lines 1 to 6). Furthermore, Tris-HCl's use for washing in the method of document D2 was advantageous because it did not require a change of the buffer for the subsequent elution step under alkaline conditions.

Admission of auxiliary requests 9 to 11 filed at the oral proceedings into the appeal proceedings

Auxiliary requests 9 to 11 should not be admitted into the appeal proceedings. The argument that no technical effect was achieved across the whole breadth of claim 1 by the pH difference of 0.5 compared to document D2 was already raised by appellant II during the first instance proceedings (see page 8, second paragraph of the decision under appeal, and page 3, second paragraph of the minutes). Moreover, this argument was maintained in appellant II's statement of grounds of appeal (see page 23, third paragraph to page 24, fifth paragraph). Lastly, the limitation of the method of claim 1 in auxiliary requests 9 to 11 to an elution "*in the presence of an elution buffer, wherein the second pH is within a buffering range of the elution buffer*", did not distinguish the claimed method further from that of

document D2, since all of the working examples in this document eluted the bound nucleic acids with a Tris-HCl buffer at pH 9, i.e. at a pH within Tris-HCl's buffering range (pH 7.1 to 9).

XIX. Appellant I requested that the decision under appeal be set aside and the patent be maintained on the basis of one of auxiliary requests 6 to 8 filed with the letter dated 16 September 2020, or alternatively, on the basis of one of auxiliary requests 9 to 11 submitted during the oral proceedings. Appellant I further requested that documents D13 to D16 not be considered in the appeal proceedings.

XX. Appellant II requested that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Admission of documents D13 to D16 into the appeal proceedings

1. The board sees no reason to overturn the opposition division's decision to admit documents D13 to D16 into the proceedings. These and other documents were submitted by both parties only a few days prior to the oral proceedings before the opposition division. The opposition division "*decided to admit all of these documents, since they were considered important to establish common practise in the field regarding inclusion of hazardous agents in nucleic acid isolation kits*", and based its decision in part on these documents, after hearing the parties on this issue (see decision under appeal, points 2.3.3.2.1 and 2.3.3.2.2, Minutes, points 14 to 16). In the board's opinion the opposition division may establish, based on documentary evidence, what was common practise for the skilled

person at a particular date. It has the discretion to take into account this evidence according to Article 114(2) EPC, even if the evidence is late filed. In this context, *prima facie* relevance of late filed documents is one of the admission criteria to be considered.

2. The admission of documents D13 to D16 was thus not incorrect. The EPC does not provide an express legal basis for excluding in appeal proceedings documents which were admitted into the first-instance proceedings. At least if the opposition division correctly used its discretion to admit the documents and based the decision under appeal on them (see e.g. T 1852/11, Reasons 1.3), as was done in the present case, the Board sees no reason to exclude them from the appeal proceedings.

Auxiliary request 6

Claim interpretation - claim 1

3. Claim 1 is directed to a method for isolating a nucleic acid comprising at least a binding, a washing and an elution step as defined in steps (i) to (iii). Claim 1 further specifies that "*the wash solution comprises a buffer with a buffering range that encompasses a pH that is higher than the first pH* [i.e. "*an acidic pH*" applied for binding the nucleic acid to a solid phase, comment added by the board], *and the wash solution is at a pH that is within a buffering range of the binding buffer but lower than the buffering range of the buffer of the wash solution, and wherein the pH of the wash solution is pH 6.0 or less*".

4. Thus, claim 1 defines for the wash buffer various pH-related requirements, which affect the binding buffer cited in step (i) of claim 1 too:

4.1 The wash buffer's buffering range as defined in step (ii) of claim 1 has to encompass at least one pH value that is higher than the "*acidic*" pH used for the binding buffer in step (i) of claim 1. A definition of "*acidic*" is not given in the patent. According to the term's common meaning in the art, a pH below 7.0 is generally considered as acidic, a pH above 7.0 as alkaline, while pH 7.0 is neutral. Accordingly, claim 1 encompasses wash buffers with "acidic" and "alkaline" buffering ranges (i.e. their buffering range is either below or above pH 7.0) that include either the "*acidic pH*" of the binding buffer, or not. It has not been contested that the term "*buffering range*" in claim 1 describes the buffer's pH range wherein added acids and bases are effectively neutralised, while a relatively constant pH is maintained. In other words, the term relates to the buffer's effective buffering capacity.

4.2 The wash buffer must further be used at a pH which lies **within** the "*buffering range*" of the binding buffer, but **below** its own buffering range, wherein the highest pH is 6.0 or lower.

4.3 The buffering range of the binding buffer is not defined in claim 1, except that it must comprise the pH used for the wash buffer. The binding buffer is used at an "*acidic*" pH, which in the absence of any definition, lies within or outside of the buffer's own buffering range. Moreover, according to step (iii) of claim 1, the binding buffer must be used at a pH that is lower than pH 6.5.

4.4 The elution of the bound nucleic acids as defined in step (iii) of claim 1 is performed in the pH range "*6.5-10*", which has to be higher than the pH used for the binding buffer in step (i). The type of solvent used for eluting the nucleic acids is not defined in step (iii). Hence, water or buffers may be used. Furthermore, since the buffering range of the wash buffer is not defined in claim 1 in relation to the solvent used for elution, the claim encompasses wash buffers with buffering ranges that overlap with the pH of the solvent used for elution, or not.

4.5 Accordingly, claim 1 comprises various combinations of binding buffers, wash buffers, and solvents for eluting the bound nucleic acids, comprising buffers or water. For illustration purposes only, two examples of such buffer combinations falling within claim 1 are given in the following to demonstrate the interrelationship of the various parameters defined in steps (i) to (iii) of claim 1:
An **acetate** binding buffer (buffering range: pH 3.6 to 5.6) can be used between **pH 1.0 < 5.9** (i.e. all pHs are "*acidic*", below pH 6.5, and lower than the upper limit of pyridine's buffering range), if combined with a **pyridine** wash buffer (buffering range: pH 4.9 to 5.9) used between **pH 3.6 and 4.8** (i.e. all pHs are ≤ 6.0, lie within the buffering range of the **acetate** binding buffer, but below **pyridine's** buffering range);
a **citrate** binding buffer (pK2, buffering range: 3.0 to 6.2) can be used at **pH 1 < 5.6** (i.e. all pHs are "*acidic*", below pH 6.5, and lower than the upper limit of acetate's buffering range), if combined with an **acetate** wash buffer (buffering range: pH 3.6 to 5.6) when used between **pH 3.0 and 3.5** (i.e. all pHs are ≤ 6.0, lie within **citrate's** buffering range, but below **acetate's** buffering range). The buffering ranges of the

exemplary buffer combinations indicated above are disclosed in Table 1 on pages 3 to 5 of the patent.

The elution step for both examples can be performed by any buffer or water at a pH between 6.5 to 10.

4.6 Claim 1 further excludes from the method the presence of chaotropic agents and organic solvents.

Novelty

5. At the oral proceedings, the board concluded that auxiliary request 6 was novel over the disclosure of document D2. In view of the finding that the method of claim 1 of auxiliary request 6 lacks an inventive step (see below), the board sees in the present case no merit in providing a detailed reasoning as to why the method of claim 1 is considered novel.

Inventive step

Closest prior art and technical problem

6. Document D2 is one of the documents cited by both parties as closest prior art for the method of claim 1.

7. Document D2 discloses a method for isolating nucleic acids from liquid samples based on solid carriers (see column 1, lines 4 to 6). The binding of the nucleic acids to the solid material is performed under acidic conditions in the range of pH 1 to 6. The method uses further a wash step, likewise performed under acidic conditions (≤ pH6.5), followed by an elution step of the nucleic acids from the carrier under alkaline conditions in the range of pH 7.5 to 9 (see column 2, lines 5 to 10, 31 to 35).

7.1 Document D2 discloses in the context of binding that the binding is "*carried out by means of simple lowering of the pH to below pH 6. For this, a binding buffer is used which can maintain a pH range from 1 to 6, preferably from 3 to 5. As is known, the purine bases of the nucleic acids have improved stability in the preferred pH range. Suitable buffers are, for example, formate, acetate, citrate buffers or other buffer systems which have adequate buffer capacity in the pH range mentioned*" (see column 3, lines 11 to 18).

7.2 Regarding the washing, document D2 mentions that the "*carrier material with the nucleic acids bound thereto can be washed with the described binding buffer or with other phosphate-free buffers, where the pH of this buffer should be between 4 and 7, preferably between 4.5 and 6.5*", and that the "*wash buffers disclosed according to the invention do not elute the bound nucleic acids even if these have been adsorbed on the carrier material with addition of chaotropic substances. In a wash buffer according to the present invention, additions of organic solvents and/or chaotropic substances are not necessary in order to avoid the premature elution of nucleic acids*" (see column 3, lines 36 to 51).

7.3 As regards the elution of the bound nucleic acids, document D2 reports that "*it has been found that the nucleic acid purified in this way can be dissolved from the solid phase by a simple increase in the pH to above 7.5. The elution buffer used for this should maintain a pH range from 7.5 to 9, preferably 8 to 8.5. Suitable buffers are, for example, tris HCl buffer, tricine, bicine and other buffers which buffer in this pH range, preferably tris/HCl*" (see column 3, lines 60 to 65).

7.4 In summary, the generic concept underlying the nucleic acid isolation method of document D2 is that the binding and washing steps are performed under acidic conditions (preferably in the pH ranges of 3 to 5, and 4.5 to 6.5, respectively), which cause a stable binding of the nucleic acids to the solid material, while the nucleic acids are eluted under alkaline conditions (preferably in the pH range of 8 to 8.5). The method does not use any organic solvents and chaotropic agents, since a simple pH switch between acidic and alkaline conditions is sufficient for obtaining high yields of isolated nucleic acids. While document D2 reports that for achieving this purpose the binding and elution buffers should "*maintain*" the pH in the respective ranges indicated above, i.e. show an "*adequate buffer capacity*" or buffering range, no such maintenance is mentioned in the context of washing which is performed at a pH within the ranges indicated above.

7.5 Examples 1 to 3 of document D2 disclose the exclusive use of Tris-HCl as buffer for washing and eluting the bound nucleic acids at pHs 6.5 and 9, respectively (see Table in Example 1 of column 4). It is uncontested that the Tris-HCl buffer was intentionally selected in document D2 for these purposes. The use of Tris-HCl allows, after washing, the direct elution of the bound nucleic acids by switching the pH from 6.5 to 9 without changing the buffer, since pH 9 lies within Tris-HCl's buffering range of pH 7.1 to 9. Therefore, Tris-HCl is indicated in document D2 as the preferred elution buffer (see point 7.3 above).

7.6 Example 3 of document D2 further discloses that the yield of the isolated nucleic acids strongly depends on

the pH used for the binding reaction (see the Table in column 6). A maximum yield of 90% is obtained at pH 4.5, which decreases stepwise by 10% after increasing the binding by 0.5 pH steps until pH 5.5 (70% yield) is reached. A yield drop to 50% is observed if the binding is performed at pH 6.0, falling to a 20% yield at pH 6.5, and a 2% yield at pH 7.0. Example 3 thus demonstrates, in line with the generic concept indicated above, that nucleic acids bind to a solid carrier material at an acidic pH (pH 4.5 to 5.5), while significantly less nucleic acids bind under weak acidic conditions (pHs 6.0, or 6.5), or almost none under neutral pH binding conditions (pH 7.0).

7.7 Example 3 discloses different binding buffers: Acetate/KOH and Acetate/NaOH (buffering range: pH 3.6 to 5.6), MES (buffering range: pH 5.5 to 6.7), and Tris-HCl (buffering range: pH 7.1 to 9.0). Tris-HCl at pH 7.0 as binding buffer is used as a control.

7.8 The acetate binding buffer embodiments of Example 3 of document D2 differ from the method of claim 1 in two features. Firstly, in that the Tris-HCl wash buffer's pH 6.5 lies not within the buffering range of the acetate binding buffer (see point 7.7), and secondly in that the pH of the wash buffer is not pH 6.0 or lower. Despite these two differences the yield of nucleic acids by the acetate binding buffer embodiments of Example 3 is 90% and 80%, depending on the pH used for binding (pHs 4.5 and 5.0, respectively).

7.9 The MES binding buffer embodiment of Example 3 in document D2 differs from the method of claim 1 in one feature only, i.e. the use of the Tris-HCl wash buffer at pH 6.5 instead of pH 6.0 or lower. The overall yield of nucleic acids obtained by the MES binding buffer

embodiment is 70% or 50%, depending on the binding pH used (pHs 5.5, and 6.0, respectively). The yield is thus lower than that for the acetate binding buffer embodiments. This demonstrates the importance of a low acidic pH for the binding reaction, because the overall conditions between the acetate and MES binding buffer embodiments of Example 3 differ in a single feature only: the binding pH (pH 4.5 or 5.0, instead of pH 5.5 or 6.0, respectively).

7.10 The MES binding buffer embodiment of Example 3 in document D2 represents, in line with the established case law, the closest prior art for the claimed method since it differs in one feature only (see Case Law, I.D.3.1.). This embodiment will be considered in the following.

8. In order to formulate the objective technical problem underlying the subject-matter of claim 1, the effect of the distinguishing feature (here: a 0.5 pH difference of the wash buffers) with respect to the MES binding buffer embodiment of document D2 must be determined.

9. It is established case law that the technical problem has to be determined on the basis of the effect actually achieved by the distinguishing feature vis-a-vis the closest prior art. Moreover, this effect has to be achieved over the entire area claimed. If this is not the case, the problem must be reformulated. The submission of comparative tests is not absolutely necessary in support that the claimed effect has indeed been achieved over the entire range covered by a claim, since it suffices that this is credible on the basis of facts derived from the patent taking the skilled person's common general knowledge into account (see Case Law, I.D.4.1, I.D.4.3.).

10. Comparative data of the claimed method with the MES binding buffer embodiment of document D2 under consideration have not been submitted by appellant I. Accordingly, the question arises whether based on facts derivable from the patent it is credible that the use of a wash buffer at pH 6.0, i.e. at a pH 0.5 lower, results in the provision of a method wherein all of the different binding buffers, different wash buffers and different combinations of binding and wash buffers falling within claim 1 still obtain a high yield, as stated by appellant I.

11. As mentioned above, the MES binding buffer embodiment of document D2 achieves an absolute yield of 70% or 50% of isolated nucleic acids at a binding pH of 5.5 or 6.0, respectively.

12. The term "*high yield*" used by appellant I is relative in the absence of a reference value, since depending on the circumstances absolute yields, for example, in between 10% and 100% may be regarded as high.

13. The patent, including Examples 1 to 4, is silent on a reference value for the yield obtained by the various embodiments falling within the claimed method. The patent discloses merely a relative yield of the isolated RNA molecules in the different working examples (see e.g. Figures 1 and 3). This means that the absolute yield obtained by the various binding/wash buffer combinations, for example, in Examples 1 and 3 of the patent cannot be determined individually. Nor can the yield be compared between the combinations of binding/wash buffers in the different working examples of the patent either. This was not contested by appellant I. In other words, based on the evidence

derivable from the patent, it cannot be established whether or not the different combinations of binding/ wash buffers in Examples 1 and 3 of the patent achieve a comparable "*high yield*" under all of the conditions tested, since the information about the absolute yield is lacking in all working examples.

14. In this situation the yield can neither be compared between the claimed method and the MES binding buffer embodiment of document D2, nor can it be assessed whether the claimed method achieves a "*high yield*" over all of the binding/wash buffer combinations that fall within claim 1. Based on the experimental evidence provided in the patent, it can be established only that the claimed method isolates nucleic acids, albeit at an unknown efficiency, i.e. it remains unknown whether the yield is high or low.

15. Appellant I submitted that the experimental data in Examples 1, 3 and 4 supported an overall improvement of the claimed method across the whole range claimed over the disclosure of document D2.

16. For the reasons outlined above, the board does not agree, since based on the evidence disclosed in the patent and in the absence of an absolute yield, it cannot be credibly established that the claimed method achieves a high yield of isolated nucleic acids across the whole breadth of claim 1.

17. Appellant I further submitted that Example 4 provided comparative data that the method of the invention provided comparable high yields without using hazardous ingredients, such as chaotropic agents and organic solvents. The board is not convinced by this argument either, since the closest prior art method in document

D2 does likewise not use chaotropic agents and organic solvents. Furthermore, Example 4 provides a comparative test with an unrelated commercial product of unknown composition only. Accordingly, the results of Example 4 are irrelevant for deciding whether or not a technical effect can be ascribed to the distinguishing feature outlined above.

18. Appellant I further submitted that document D2 disclosed solely a single combination of a binding buffer and wash buffer that obtained a high yield, i.e. the acetate binding buffer embodiments. The board does not agree, since, as set out above, based on the evidence derivable from the patent, it cannot be credibly established that the claimed method achieves a high yield, either for a single combination of binding/ wash buffers, let alone for all of the combinations falling within the whole breadth of the claim.

19. In view of the considerations above, the board is not convinced that the distinguishing feature is associated with an advantageous technical effect, at least not across the whole range covered by claim 1.

20. Accordingly, a less ambitious technical problem has to be defined, which is the provision of an alternative method for the isolation of nucleic acids.

21. In view of the experimental data in Examples 1, 3 and 4 of the patent, the board is satisfied that the method of claim 1 solves this problem.

Obviousness

22. It remains to be assessed whether or not the skilled person starting from the MES binding buffer embodiment

in Example 3 of document D2, and faced with the technical problem identified above, would have arrived at the claimed method in an obvious manner.

23. Appellant I submitted that the skilled person had no motivation to further lower the pH of the Tris-HCl wash buffer to pH 6.0 or lower, since pH 6.0 instead of 6.5 was even further away from Tris-HCl's buffering range.

24. The board does not agree. Document D2 reports in column 3, lines 37 to 40 that the washing can be done either by the "*described binding buffer or with other phosphate-free buffers, where the pH of this buffer should be between 4 and 7, preferably between 4.5 and 6.5*". The skilled person knows that Tris-HCl is a phosphate-free buffer, and as set out above, document D2 mentions that this buffer is preferably used for washing and eluting purposes. Thus starting from the Tris-HCl wash buffer at pH 6.5 in Example 3 and looking for alternative wash conditions, the skilled person would have taken into consideration all of document D2's teaching for this purpose, in particular parameters that have been explicitly mentioned as preferred, such as the use of a washing pH across the whole range of "*4.5 and 6.5*" (see column 3, line 40).

25. The skilled person would have only refrained from applying this teaching if reasons for doing so were apparent. Appellant I submitted that the lowering of the pH from 6.5 to 6.0 would have removed Tris-HCl even further away from its buffering range of pH 7.1 to 9 which harbored the risk that the pH for washing became unstable. This is, however, not convincing. Tris-HCl's pH of 6.5 as disclosed for washing in Example 3 of document D2 lies already outside of it's buffering range. Since Tris-HCl cannot therefore buffer at pH

6.5, it can neither buffer at pH 6.0 too. This means that pH stability cannot be an issue in the present case, as long as the pH remains acidic during the washing to prevent a premature elution of the bound nucleic acids from the solid material (see document D2, column 3, lines 45 to 47). A condition certainly achieved by lowering the pH of Tris-HCl to 6.0.

26. The board therefore concludes that the method of claim 1 is obvious for the skilled person based on the teaching of document D2 alone. Auxiliary request 6 therefore lacks an inventive step (Article 56 EPC).

Auxiliary requests 7 and 8

27. Claims 1 of auxiliary requests 7 and 8 differ from claim 1 of auxiliary request 6 in that the feature "*wherein the second pH is within the buffering range of the wash buffer*" has been added. Claim 1 of auxiliary request 8 is further amended compared to claim 1 of auxiliary request 7, in that the feature "*pH 6.5-10*" has been replaced by "*pH 7-9*" in step (iii).

Inventive step

28. The additional/amended features in claims 1 of auxiliary requests 7 and 8 do not further distinguish the claimed methods from the disclosure of the MES binding buffer embodiment in Example 3 of document D2. The Tris-HCl buffer in Example 3 is used at pH 9 for eluting the bound nucleic acid from the solid carrier. This pH value for elution (i.e. the "*second pH*" according to claim 1) lies within Tris-HCl's buffering range of pHs 7.1 to 9, i.e. of the buffer that has been likewise used for washing. Furthermore, an elution at pH 9 as disclosed in Example 3 of document D2 falls

within the pH range cited in step (iii) of the claimed methods.

29. Accordingly, the reasons set out above under lack of inventive step for the method of claim 1 of auxiliary request 6 apply *mutatis mutandis* to the methods of claims 1 of auxiliary requests 7 and 8 (Article 56 EPC).

Admission of auxiliary requests 9 to 11 filed at the oral proceedings into the appeal proceedings

30. Since the summons to the oral proceedings were notified on 15 April 2020, Article 13 RPBA 2020 is to be applied for questions regarding any amendment to a party's appeal case (Article 24(1) RPBA 2020).

31. Appellant I filed auxiliary requests 9 to 11 during the oral proceedings in reply to an allegedly new argument raised by the board during the oral proceedings.

32. Auxiliary requests 9 to 11 represent an amendment to appellant I's case under Article 13(2) RPBA 2020 according to which any amendment to a party's appeal case made after notification of a summons to oral proceedings shall, in principle, not be taken into account unless there are exceptional circumstances, which have been justified with cogent reasons by the party concerned.

33. Auxiliary requests 9 to 11 are identical to auxiliary request 6, except that the method of claim 1 of auxiliary request 9 was limited to an elution in the presence of an elution buffer at a pH ("*second pH*") that was within the buffering range of the elution

buffer. Moreover, all claims relating to a kit have been deleted.

Claim 1 of auxiliary request 10 compared to claim 1 of auxiliary request 9 was further limited in that "*the second pH*" (i.e. the elution pH) was within the buffering range of the wash buffer too. In other words, the elution pH was within the buffering ranges of the buffers used for washing and eluting.

Lastly, claim 1 of auxiliary request 11 compared to claim 1 of auxiliary request 10 was further limited in that "*the second pH*" (i.e. the elution pH) was "*in the range of pH 7-9*", instead of "*pH 6.5-10*".

34. Auxiliary requests 9 to 11 constitute an attempt to overcome an allegedly new objection under Article 56 EPC raised by the board during the oral proceedings. It concerned the board's question whether or not it was credible based on the evidence in the patent and in light of the closest prior art method that the technical problem formulated by appellant I (see above) was solved across the whole breadth of claim 1 for the embodiment of the claimed method using strong acidic binding and wash buffers, in combination with water for eluting the bound nucleic acids from the solid material.

35. The objection that no advantageous technical effect is achieved by the distinguishing feature of claim 1 compared to the closest prior art method of document D2 over the whole range claimed was raised by appellant II already during the written phase of the opposition proceedings, and was maintained in their statement of grounds of appeal (see submission dated 12 October 2015, on page 7, paragraphs 1 to 4; statement of

grounds of appeal, page 28, last paragraph to page 29, second paragraph). Although the opposition division did not find this objection convincing and acknowledged inventive step in the decision under appeal, the board's provisional opinion deviated from this finding, at least in so far as the board was not convinced that the technical effect of a high yield was obtainable across the whole scope of claim 1 (see point 39 of the board's communication). Furthermore, point 38.4 of the board's communication mentions that claim 1 encompasses the combination of acidic wash buffers and water for elution purposes, and that in view of the evidence provided in Example 1 of the patent the achievement of a high yield across the whole scope of claim 1 appeared doubtful. Moreover, the fact that the method of claim 1 encompassed the combination of acidic binding and wash buffers was likewise mentioned in the board's communication (see point 21.3).

36. In light of these considerations, the board is not convinced by appellant I's submission that auxiliary requests 9 to 11 were filed to address a new argument of the board raised for the first time during the oral proceedings.

37. Irrespective of the above, the amendments in claims 1 of auxiliary requests 9 to 11 do not have the effect that the technical problem defined by appellant I for the method of claim 1 (see above) is solved across the whole range claimed. This is so because the fundamental issue remains as set out above, namely that based on the data in the patent it cannot be established that a high yield is obtained by all combinations of binding/ wash and elution buffers encompassed by claim 1. In the absence of an absolute yield or a reference yield, the combinations of various buffers and their effect on the

yield can neither be determined nor compared. Accordingly, an advantageous effect cannot be ascribed to the distinguishing feature of the claimed methods vis-a-vis the MES binding buffer embodiment of document D2, and hence the technical problem underlying the claimed method remains, as for the higher ranking auxiliary requests above, the provision of an alternative method for isolating nucleic acids.

38. The amendments in claims 1 of auxiliary requests 9 to 11 do also not further distinguish the claimed method over the MES binding buffer embodiment of document D2. This embodiment uses a Tris-HCl buffer at pH 9.0 for elution, i.e. "*the second pH*", which lies within the buffering range (pH 7.1 - 9.0) of the elution buffer. Moreover, pH 9.0 of the Tris-HCl buffer used in the MES binding buffer embodiment for elution purposes lies within the elution pH range cited in step(iii) of the claimed methods.

39. Consequently, exceptional circumstance according to Article 13(2) RPA 2020 do not apply in the present case. Moreover, it is not apparent that the amended claimed methods overcome *prima facie* the objections raised above under Article 56 EPC against the method of claim 1 of auxiliary requests 6 to 8. The board therefore decided not to admit auxiliary requests 9 to 11 into the appeal proceedings.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated