Internal distribution code:

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

Datasheet for the decision of 12 June 2023

Case Number: T 2035/17 - 3.3.08

Application Number: 04779084.5

Publication Number: 1651761

IPC: C12N15/10

Language of the proceedings: EN

Title of invention:
METHOD FOR ISOLATING SMALL RNA MOLECULES

Patent Proprietor:
Life Technologies Corporation

Opponent:
QIAGEN GmbH

Headword:
Isolation small RNA molecules/LIFE TECHNOLOGIES CORPORATION

Relevant legal provisions:
EPC Art. 123(2)
RPBA Art. 12(4)

Keyword:
Main request, auxiliary requests 1 to 7 - added subject-matter (yes);

Decisions cited:

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

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 2035/17 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 12 June 2023

Appellant: (Patent Proprietor)	Life Technologies Corporation 5823 Newton Drive Carlsbad, CA 92008 (US)
Representative:	HGF HGF Limited 1 City Walk Leeds LS11 9DX (GB)
No more party: (Opponent)	QIAGEN GmbH Qiagen Str. 1 40724 Hilden (DE)
Representative:	König Szynka Tilmann von Renesse Patentanwälte Partnerschaft mbB Düsseldorf Mönchenwerther Straße 11 40545 Düsseldorf (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 19 July 2017 revoking European patent No. 1651761 pursuant to Article 101(3)(b) EPC**

Composition of the Board:

Chairman	T. Sommerfeld
Members:	P. Julià
	A. Bacchin

Summary of Facts and Submissions

I. European patent no. 1 651 761 is based on European patent application no. 04 779 084.5, originally filed under the Patent Cooperation Treaty (PCT) and published as International patent WO 2005/012523 (hereinafter "the patent application"). The patent was granted with 15 claims.

II. An opposition was filed on the grounds of Article 100(a) EPC for lack of novelty and inventive step (Articles 54 and 56 EPC), Article 100(b) EPC for insufficiency of disclosure (Article 83 EPC), and Article 100(c) EPC for added subject-matter (Article 123(2) EPC). The opposition division considered the main request and auxiliary request, both filed with submissions dated 29 March 2017, to contravene Article 123(2) EPC, and the patent was revoked.

III. An appeal was lodged by the patent proprietor (appellant). In the statement setting out their grounds of appeal, the appellant filed a new main request and auxiliary requests 1 to 7. The opponent replied thereto and argued that all of the appellant's claim requests were late filed and none of them met the requirements of the EPC. Oral proceedings were requested by both parties as an auxiliary measure.

IV. By letter dated 9 October 2019, the opponent withdrew the opposition against the patent and is thus no longer a party to the appeal proceedings.

V. The appellant was summoned to attend oral proceedings scheduled for 6 October 2020.

VI. With submissions dated 21 April 2020, the appellant withdrew the request for oral proceedings and requested that the board take a decision in writing on the basis of their statement of grounds of appeal.

VII. The board cancelled the oral proceedings.

VIII. The **main request** contains fifteen claims, wherein claim 1 reads as follows:

"1. A method for isolating RNA including small RNA molecules of 100 nucleotides or fewer from a sample containing cells, the method consisting of:

a) lysing the cells in a lysing solution to produce a lysate wherein the lysing solution comprises guanidinium wherein a lysate with a concentration of at least about 1 M guanidinium is produced; and extracting small RNA molecules from the lysate with an extraction solution comprising phenol;

b) adding an ethanol solution to the lysate to result in a lysate/ethanol mixture, wherein the concentration of alcohol in the mixture is between about 35% and 70% and wherein the final guanidinium concentration is between 1 and 2.7 M

c) applying the mixture to a solid support comprising silica wherein small RNA molecules bind to the solid support and optionally washing the solid support;

d) eluting RNA including small RNA molecules of 100 nucleotides or fewer from the solid support **with an ionic solution**; and

e) using or characterizing the RNA molecules."

The emphasis, added by the board, indicates a feature not present in the main request before the opposition division and underlying the decision under appeal. This feature was added in the main request filed in the appeal proceedings.

IX. Claim 1 of **auxiliary request 1** reads as claim 1 of the main request, except for the presence of an additional method step, namely new step e), after the elution step d), as well as the deletion of the term "and" in said step d) and the renaming of the last step of the claimed method as step f). Step e) of this auxiliary request reads as:

"... e) capturing the small RNA molecules; and"

X. Claim 1 of **auxiliary request 2** reads as claim 1 of the main request, except for steps a) and b) which read as follows:

"a) lysing the cells in a lysing solution to produce a lysate wherein the lysing solution comprises guanidinium wherein a lysate with a concentration of about 4 M guanidinium is produced; and extracting small RNA molecules from the lysate with an extraction solution comprising phenol;

b) adding an about 100% ethanol to the lysate to result in a lysate/ethanol mixture, wherein the concentration of alcohol in the mixture is between about 35% and 70%"

XI. Claim 1 of **auxiliary request 3** reads as claim 1 of auxiliary request 1, except for steps a) and b) which read as in auxiliary request 2.

XII. Claim 1 of **auxiliary request 4** reads as claim 1 of auxiliary request 2, except for step b) which reads as follows:

"b)adding an about 100% ethanol to the lysate to result in a lysate/ethanol mixture, wherein the concentration of alcohol in the mixture is 35%, 45%, 50%, 55%, 60%, 65% or 70%"

XIII. Claim 1 of **auxiliary request 5** reads as claim 1 of auxiliary request 3, except for step b) which reads as in auxiliary request 4.

XIV. Claim 1 of **auxiliary request 6** reads as claim 1 of auxiliary request 4, except for step b) which reads as follows:

"b) adding an about 100% ethanol to the lysate to result in a lysate/ethanol mixture, wherein the concentration of alcohol in the mixture is 55%"

XV. Claim 1 of **auxiliary request 7** reads as claim 1 of auxiliary request 5 except for step b) which reads as in auxiliary request 6.

XVI. Appellant's arguments, insofar as they are relevant to the present decision, may be summarised as follows:

Consideration/admission of the new claim requests

The feature "with an ionic solution" introduced into step d) of claim 1 brought the wording of this claim more closely in line with that of claim 48 of the patent application. The amendments introduced into auxiliary requests 1 to 7 were made in response to

objections under Article 123(2) EPC raised for the first time at the oral proceedings before the first instance.

Main request - Article 123(2) EPC

The generic features recited in claim 48 of the patent application were narrowed in accordance with the specific disclosures of the examples of the patent application, in particular Example 1, which directly and unambiguously disclosed a method having the specific combination of features present in claim 1 of the main request.

Ethanol was disclosed as a preferred alcohol solution on page 6, lines 29 and 30 and as shown in Example 1 of the patent application. Guanidinium was already mentioned in step a) of claim 48 and, on page 5, lines 24 to 29 of the patent application, it was stated that "the concentration of a chaotropic agent in the solutions of the invention, particularly lysing solutions, is about, is at most about, or is at least about 0.5, 0.6, ... 3.4, 3.5 M or more, and ranges therein". The use of the expression "and ranges therein" was a widely-accepted convention of the English language adopted to avoid the need for lists of excessive lengths where exactly the same information could be conveyed in a more concise fashion. Without the use of such conventions, in respect of this single list, it would have been required to include a passage with a list of all possible ranges resulting from using all these specific values. One of all these ranges expressly contemplated was the range 1.0 to 2.7 M. This range was thus directly and unambiguously derivable from the patent application.

Auxiliary request 1

Auxiliary request 1 was identical to the main request, except that step e) "capturing the small RNA molecules" was added into claim 1. Basis for this amendment could be found in claim 48 of the patent application.

Auxiliary request 2

Basis for amending the guanidinium concentration in step a) to about 4 M was found on page 5, lines 27 to 29 of the patent application. In line with the case law, the disclosure of the guanidinium range of between about 2.0 M and 4 M constituted a direct and unambiguous disclosure of the two specifically named end-points, i.e. about 2.0 M and about 4.0 M. Basis for specifying that the (added) ethanol was about 100% ethanol in step b) could be found at page 6, lines 17 to 30 of the patent application, wherein ethanol was also specifically contemplated for use in aspects of the invention. A guanidinium concentration of about 4 M and an (added) ethanol concentration of about 100%, (absolute ethanol) were consistently and successfully used in combination to isolate small RNA molecules throughout the examples of the patent application using a method consisting of the steps recited in claim 1.

The feature "and wherein the final guanidinium concentration is between 1 and 2.7 M" present in step b) of claim 1 of the main request would have been redundant in step b) of claim 1 of auxiliary request 2 and therefore, this feature was deleted. In the context of a method "consisting" of the steps recited in claim 1, when about 4M guanidinium was used in step a) and an ethanol solution of about 100% was added in step b), inevitably resulted in a final guanidinium

concentration of between 1 and 2.7 M in the lysate/ ethanol mixture.

Auxiliary request 3

This auxiliary request was identical to auxiliary request 1, except for the amendments introduced into steps a) and b) of claim 1 that read as in auxiliary request 2. Thus, the basis in the patent application was the same as that given for auxiliary requests 1 and 2.

Auxiliary request 4

Claim 1 of auxiliary request 4 was identical to claim 1 of auxiliary request 2 except that the concentration range of alcohol in the mixture of step b) was amended to the discrete values "35%, 45%, 50%, 55%, 60%, 65% or 70%". Basis for these values was found in Example 1 of the patent application. A guanidinium concentration of about 4 M and an ethanol concentration of about 100% (absolute ethanol) were consistently and successfully used in combination in Example 1 to generate a final ethanol concentration in the lysate/alcohol mixture of the discrete values recited in the claim for isolating small RNA molecules in the context of a method consisting of the steps recited in claim 1.

Auxiliary request 5

Claim 1 of auxiliary 5 request was identical to claim 1 of auxiliary request 3, except that the concentration range of alcohol in the mixture of step b) was amended to the discrete values recited in auxiliary request 4, namely "35%, 45%, 50%, 55%, 60%, 65% or 70%". Thus, the

basis in the patent application was the same as that given for auxiliary requests 3 and 4.

Auxiliary request 6

Claim 1 of auxiliary request 6 was identical to claim 1 of auxiliary request 4, except that the concentration of alcohol in the mixture of step b) was amended to 55%. Basis for the amendment and this discrete value could be found in Example 1 and on page 6, lines 27 and 28 of the patent application.

Auxiliary request 7

This auxiliary request was identical to auxiliary request 5, except that the concentration of alcohol in the mixture of step b) was amended as in auxiliary request 6. Thus, the basis in the patent application was the same as that given for auxiliary requests 5 and 6.

XVII. The appellant (patent proprietor) requested that the decision under appeal be set aside and that the application be remitted to the department of first instance for further prosecution on the basis of the main request or, in the alternative, of any of auxiliary requests 1 to 7 in that order.

Reasons for the Decision

Consideration/admission of appellant's new claim requests

1. The main request and auxiliary requests 1 to 7 were filed by the appellant with the statement setting out their grounds of appeal. None of these requests were

examined by the opposition division at the first instance proceedings.

2. In the statement of grounds of appeal, the appellant provided reasons to explain the filing of these requests in appeal proceedings.

2.1 As regards the main request, the feature in step d) of claim 1, namely "with an ionic solution", was introduced into claim 1 "in order to more closely adhere to the language of original claim 48"; wherein at the proceedings before the opposition division, original claim 48 had been given as a basis for claim 1 (cf. page 3, last paragraph of the grounds of appeal).

2.2 As regards auxiliary requests 1 to 7, the appellant stated that "the amendments made in Auxiliary Requests 1 to 7 are responsive to specific added matter objections that were raised for the first time during the Oral Proceedings of 1 June 2017" (cf. page 2, tenth paragraph of the grounds of appeal). And, with reference to the opposition division's preliminary opinion, the appellant further stated that "[t]he auxiliary requests provided herewith are filed in direct response to the new interpretation taken by the OD at the Oral Proceedings with regard to the teaching of the application as filed" (cf. page 3, third paragraph of the grounds of appeal).

3. In view of the reasons provided below with regard to allowability of these requests, the question of admittance of these requests can be left aside.

Main request
Article 123(2) EPC

4. Except for the feature "with an ionic solution" in step d) of claim 1, this claim is identical to claim 1 of the main request underlying the decision under appeal. The opposition division considered that the subject-matter of claim 1 of the then main request had no basis in the patent application; in particular, the feature in step b) of claim 1, namely "the final guanidinium concentration is between 1 and 2.7 M", was considered not to find basis in the disclosure on page 5, lines 18 to 30 of the patent application, Example 9 (Figures 3 to 6), and claim 48 of the patent application. Therefore, claim 1 was not in compliance with Article 123(2) EPC (cf. page 4, point 3.3.4 to page 6, point 3.3.15 of the decision under appeal).

5. Claim 48 of the patent application, from which claim 1 of the main request derives, reads as follows:

"48. A method for isolating small RNA molecules from a sample comprising:

a) lysing cells in the sample with a lysing solution comprising guanidinium, wherein a lysate with a concentration of at least about 1 M guanidinium is produced;

b) extracting small RNA molecules from the lysate with an extraction solution comprising phenol;

c) adding to the lysate an alcohol solution for [sic] form a lysate/alcohol mixture, wherein the concentration of alcohol in the mixture is between about 35% to about 70%;

d) applying the lysate/alcohol mixture to a mineral or polymer support;

e) eluting the small RNA molecules from the mineral or polymer support with an ionic solution;

f) capturing the small RNA molecules; and

g) using the isolated small RNA molecules."

6. The appellant referred to the disclosure on page 5, lines 24 to 27 of the patent application disclosing a list of twenty-six specific values of guanidinium concentration, namely from between "about 0.5, 0.6, ... [up to] ... 3.4, 3.5 M or more", and in particular to the expression "and ranges therein" immediately following this list of specific values. According to the appellant, this expression is a widely-accepted convention of the English language which stands for a list that includes all possible ranges resulting from a combination of all the disclosed specific values and thus includes, among all these possible ranges, the range "between 1 and 2.7 M" (cf. page 3, point 3 *et seq.*, in particular page 5, first paragraph of appellant's grounds of appeal).

7. In view of these arguments, the following issues are relevant:

7.1 The disclosure referred to by the appellant on page 5, lines 24 to 27 of the patent application concerns the "lysing solution" used in step a) of claim 1 (cf. page 5, lines 19 to 21 and lines 27 to 31), but not the "lysate" resulting from carrying out step a) of claim 1. It refers to the guanidinium concentration of

the lysing solution used in step a) of the disclosed method but not to the guanidinium concentration of the (resulting) produced lysate. Claim 48 of the patent application refers to "a lysate with a concentration of at least about 1 M guanidinium", but not to any (guanidinium) concentration range. There is no other reference to guanidinium, let alone a concentration range thereof, in claim 48 of the patent application.

7.2 On page 7, lines 15 to 29 of the patent application, reference is made to a step of the method that is carried out "prior to applying the lysate to a solid support". This may correspond to step b) of claim 1 of the main request. In this passage, there is a disclosure of "a concentration of guanidinium that is about, at least about, or at most about 0.5, 0.6, ... [up to] ... 2.9, 3.0 M, or any range derivable therein" (cf. page 7, lines 24 to 26). This passage however cannot be a basis for step b) of claim 1 of the main request for at least the following reasons:

7.2.1 First, the expression "any range derivable therein" is a generic disclosure that might well comprise the specific range of a guanidinium concentration "between 1 and 2.7 M". However, in line with the established case law, a generic disclosure does not support, and cannot be acknowledged as a basis of, a specific disclosure (cf. Case law of the Boards of Appeal of the EPO, 10th edition 2022, in the following "Case Law", II.E.1.10).

7.2.2 Second, the passage on page 7 requires to add the guanidinium - for obtaining the indicated range of guanidinium concentration - "prior to the addition of an alcohol" (cf. page 7, line 18; see also Example 9). There is no indication of such a step in the method of

claim 1 of the main request, i.e. how and when the final guanidinium concentration mentioned in step b) is achieved/added to the lysate resulting from step a). There is no indication in claim 1 of the main request on whether the guanidinium is added before, after, or simultaneously with, the addition of the ethanol solution mentioned in step b) of claim 1. Thus, claim 1 comprises several embodiments that are not supported by the disclosure on page 7 of the patent application (cf. "Case Law", *supra*, II.E.1.3.9.e) and II.E.1.11.8).

7.2.3 Third, the ranges of guanidinium concentration disclosed on page 7 are concentrations of the "lysate after homogenization" having no relationship with the percentage of the alcohol mixture of the following step, i.e. after adding an alcohol solution such as in step c) of claim 48 of the patent application. However, in step b) of claim 1 of the main request, the concentration range of guanidinium is defined as "final", i.e. once the ethanol has been added, and thus directly related to the concentration of the alcohol mixture indicated in this claim, i.e. "between about 35% and 70%". In this context, the opposition division referred to Example 9 and the specific values of final ethanol and final guanidinium concentrations disclosed in this example (see the Table on page 5 of the decision under appeal). However, as stated by the opposition division, this disclosure might be a basis for several combinations of specific concentrations of guanidinium and ethanol but certainly not for the concentrations ranges of guanidinium and alcohol/ ethanol mentioned in claim 1 of the main request (cf. page 5, point 3.3.13, last paragraph, and page 6, point 3.3.14 of the decision under appeal).

7.3 Moreover, by using the term "consisting" in the preamble of claim 1 of the main request, the scope of this claim must be understood in a restrictive manner, i.e. as excluding the presence of additional (method) steps other than those explicitly mentioned in the claim (cf. "Case Law", *supra*, II.A.6.2 and II.E.1.15). However, claim 48 of the patent application comprises a further step f), namely "capturing the small RNA molecules", between the elution of the small RNA molecules from the mineral or polymer support and the use of the "isolated" small RNA molecules, i.e. step e) and step g), respectively. The same method is also disclosed on page 9, lines 21 to 28 of the patent application. However, neither step f) is present in the method of claim 1 of the main request nor does claim 1 require the RNA molecules of step e) - which are eluted from step d) - to be "isolated" for use or characterise them, a requirement present in the methods disclosed in claim 48 and on page 9, lines 21 to 28 of the patent application. Thus, for this reason alone, neither the method of claim 48 nor the disclosure on page 9, lines 21 to 28 of the patent application, provide a basis for the method of claim 1 of the main request.

8. Therefore, at least for these reasons given above, the subject-matter of claim 1 of the main request and thus, the main request, contravenes Article 123(2) EPC.

Auxiliary request 1
Article 123(2) EPC

9. Claim 1 of this auxiliary request differs from claim 1 of the main request by the presence of an additional method-step, namely step "e) capturing the small RNA molecules", after the step of "eluting RNA including

small RNA molecules of 100 nucleotides or fewer from the solid support with an ionic solution" and before the step of "using or characterizing the RNA molecules", i.e. in-between steps d) and f), respectively, of claim 1 of auxiliary request 1 which are identical to steps d) and e), respectively, of claim 1 of the main request.

10. This additional step e) is identical to step f) of claim 48 of the patent application. Therefore, this amendment introduced into claim 1 of auxiliary request 1 overcomes the objection raised under Article 123(2) EPC in point 7.3 above.

11. However, insofar as claim 1 of auxiliary request 1 is identical to claim 1 of the main request except for the presence of the additional step e) referred to above, claim 1 of auxiliary request 1 contains the other feature present in claim 1 of the main request that does not comply with Article 123(2) EPC, namely the feature "final guanidinium concentration is between 1 and 2.7 M" in step b) of the claimed method. Thus, the reasons given in points 7.1 and 7.2 *et seq.* above for the main request are also relevant and apply to claim 1 of auxiliary request 1.

12. Therefore, auxiliary request 1 contravenes Article 123(2) EPC.

Auxiliary request 2
Article 123(2) EPC

13. The method of claim 1 of auxiliary request 2 is also a method "consisting of" the same steps as those defining the method of claim 1 of the main request and thus it does not contain step f) of the method of claim 48 or

the method disclosed on page 9, lines 21 to 28 of the patent application, namely "capturing the small RNA molecules". Therefore, neither the method of claim 48 nor that disclosed on page 9, lines 21 to 28 of the patent application, provide a basis for claim 1 of auxiliary request 2 (cf. point 7.3 *supra*).

14. Methods for efficiently isolating small RNA molecules from cells which do not comprise an intermediate step for capturing the small RNA molecules are disclosed in the patent application, for instance on page 5, lines 4 to 9 and claim 1 of the patent application. Indeed, the intermediate capture of small RNA molecules is disclosed in the patent application as applying only to some embodiments, as stated on page 9, lines 5 and 6 of the patent application. However, these disclosures are generic with no reference to any specific lysing solution and/or alcohol solution, let alone to specific concentration and/or concentration ranges of the particular components of these solutions. Thus, none of these generic disclosures provides a basis for the specific features characterizing the method of claim 1 of auxiliary request 2.

15. In this context, it is worth noting that, according to the case law of the Boards of Appeal, the content of the patent application must **not** be considered to be **a reservoir** from which features pertaining to separate embodiments of the application could be combined in order to artificially create a particular embodiment. In the absence of any pointer to that particular combination, this combined selection of features - as such - does not, for the skilled person, emerge clearly and unambiguously from the content of the patent application (cf. "Case Law", *supra*, II.E.1.6.1.a)); even though it might well be rendered obvious by the

content of the application (cf. "Case Law", *supra*, II.E.1.3.4.a)). In the present case, the particular combination of specific features characterising the claimed method results from a combined selection of features that are disclosed in the patent application in separate embodiments and/or different levels of generalisation.

16. As regards the feature in step a) of claim 1 "a lysate with a concentration of about 4 M guanidinium is produced":

16.1 Both methods disclosed in claim 48 and on page 9, lines 21 to 28, of the patent application refer to "a lysate with a concentration of at least about 1 M guanidinium" but not to a concentration of at least about 4 M guanidinium. On page 5, lines 27 to 29 of the patent application, reference is made to specific embodiments wherein the concentration of guanidinium is "between about 2.0 M and 4.0 M". However, as stated in point 7.1 *supra*, this concentration characterises the "lysing solution" but not the (resulting) produced "lysate". It might well be that, in some cases, the concentration of guanidinium of the lysing solution is the same as that of the resulting/produced lysate but that might not necessarily and always be the case. Indeed, this depends on both the lysing conditions used as well as the material lysed, i.e. the "sample containing cells" referred to in the preamble of the claimed method. Since there is no definition or characterisation of said sample in claim 1 of auxiliary request 2, the sample comprises liquid samples for which the concentration of guanidinium in the lysing solution is not necessarily identical to that of the resulting lysate.

16.2 In fact, on page 7, lines 15 to 26 of the patent application, reference is made to the concentration of guanidinium of the lysate and to the amount of guanidinium to be added to the lysate - prior to the addition of an alcohol - for applying said lysate to a solid support. Several specific values for the guanidinium concentration of the lysate are disclosed therein, the highest value being only "about 3 M" (cf. page 7, lines 22 and 25). From page 14, line 22 to page 16, line 16, there is a disclosure concerning "Creating Cell Lysates" wherein the lysing solution is further defined. In particular, the guanidinium salts are disclosed therein as including "guanidinium hydrochloride and guanidinium isothiocyanate" and that for certain embodiments, "they may be present in a concentration of about 2 to about 5 M" (cf. page 14, lines 29 to 31). It is also disclosed that "[i]n some embodiments of the invention, a lysis solution includes: guanidinium thiocyanate, ..." (cf. page 15, lines 20 and 21). There is however no reference to a lysate characterised by the features present in step a) of claim 1 of auxiliary request 2.

16.3 According to the patent application, Example 1 "provides the basis for the invention and is referred to in the Examples as the Ambion miRNA Isolation Kit (AMIK) procedure" (cf. page 24, lines 20 and 21). The procedure described in Example 1 is also defined as "the standard procedure" (cf. page 30, line 20) and the lysing solution used therein as "the standard lysis buffer", which has a guanidinium concentration of 4 M (cf. page 27, lines 10 and 11; page 28, lines 23 to 25; page 31, line 19). It might be argued that, since in most of the examples the sample used is frozen and only the lysing solution/lysis buffer is added (cf. page 24, line 22 in Example 1; page 27, line 6 in Example 3;

page 28, line 22 in Example 4; page 29, lines 5 and 22, in Examples 5 and 6, respectively; page 31, line 3 in Example 8), the concentration of guanidinium in the resulting lysate will be essentially that of the lysing solution/lysis buffer. However, as stated above, this condition is not required in the method of claim 1 of auxiliary request 2. Moreover, there are few examples in the patent application wherein there is no indication that the samples used were frozen, such as the cells collected from two human cell lines in Example 7 (cf. page 30, lines 16 and 17) and the mouse liver used in Example 9 (cf. page 31, line 19).

16.4 In addition, whilst in all the methods described in the examples of the patent application, the guanidinium salt used in the lysing solution is GuSCN, one of the specific salts of guanidinium disclosed on page 14, lines 29 and 30 of the patent application, the method of claim 1 of auxiliary request 2 is not restricted to this specific guanidinium salt. The properties of all these salts are known in the art to be different and thus they might provide different results depending on their concentration and on other specific conditions (lysing, extraction, alcohol addition, etc.) used.

16.5 In light of these considerations, it must be concluded that this specific feature in step a) of claim 1 has no basis in the patent application.

17. As regards the feature in step b) of claim 1 "the concentration of alcohol in the [lysate/ethanol] mixture is between about 35% and 70%" which is achieved by "adding an about 100% ethanol to the lysate":

17.1 The range of alcohol concentration in step c) of the methods disclosed in claim 48 and on page 9, lines 21

to 28 of the patent application is defined as "between about 35% to about 70%". Whilst these methods refer to an "alcohol" in general, in the context of step b) of claim 1 of auxiliary request 2 the alcohol is understood to be ethanol. On page 6, lines 25 to 27, reference is also made to this range of concentration ("of about 35% to about 70%") for alcohols in general and then, immediately thereafter, ethanol is mentioned as being "specifically contemplated for use in aspects of the invention" (cf. page 6, lines 29 and 30).

17.2 Ethanol is also mentioned in all examples of the patent application but not the specific concentration range of "between about 35% and 70%". Example 6 is the sole example that, after addition of "absolute ethanol" to the lysate, refers to "final concentrations of 35, 40, 45, 50, 55, 60, 65, and 70% ethanol" (cf. page 29, lines 26 to 28). However, these are all specific values but not concentration ranges, and the term "about" is not present therein. Moreover, contrary to the method of claim 1 of auxiliary request 2, there is no reference in the procedure described in Example 6 to an (intermediate) step of "extracting [the] small RNA molecules from the lysate with an extraction solution comprising phenol", prior to the addition of the "absolute ethanol". Methods using non-alcohol organic solvent are different from those using alcohol (ethanol) solutions or using both, non-alcohol organic solvents and alcohol (ethanol) solutions (cf. page 16, lines 24 to 26).

17.3 In this context, it is worth noting that the methods disclosed in claim 48 and on page 9, lines 21 to 28 of the patent application refer to "adding to the lysate an alcohol solution" in general without defining the specific percentage of alcohol in said solution. On

page 6, lines 17 to 20 of the patent application, the alcohol solution added to the lysate is defined by several specific percentages of alcohol, namely "... at most about 5, 10, 15, ... 90, 95, or 100% alcohol, or any range therein" (cf. page 6, lines 19 and 20). Whilst in most of the methods described in the examples of the patent application, the ethanol added to the lysate is defined as "absolute ethanol" (cf. page 29, line 27 in Example 6; page 30, line 23 in Example 7; page 31, line 11 and lines 26 and 27 in Examples 8 and 9, respectively), in other examples there is no indication on the ethanol percentage of the ethanol solution added to the lysate, such as in Example 1 (cf. page 25, lines 4 and 5), or the percentages indicated are much lower than 100%, namely "40%, 50%, 60%, and 70%" in Example 3 (cf. page 27, lines 17 and 18). Thus, there is no basis in the patent application for the term "adding an about 100% ethanol" in step b) of claim 1 of auxiliary request 2.

17.4 Indeed, the volume of the alcohol (ethanol) solution added to the lysate - resulting from step a) of the claimed method - for achieving the concentration of alcohol in the lysate/ethanol mixture required in the claim ("between about 35% and 70%") depends on the alcohol (ethanol) percentage of said solution. A low/high alcohol (ethanol) percentage requires more/less volume, respectively, which thus directly affects the concentration of guanidinium present in the lysate/ethanol mixture; i.e. the mixture which is applied to the solid support in step c) of the claimed method. This might have substantive and relevant effects on the size ranges of the small RNA molecules isolated, as explicitly acknowledged in Example 9 of the patent application, namely "... by manipulating both salt and ethanol concentration the binding of quite restricted

size ranges of RNA molecules can be achieved, indicating more refined size-fractionation procedures can be performed" (cf. page 32, lines 5 to 8).

17.5 In the light of these consideration, it must be concluded that these specific features in step b) of claim 1 have no basis in the patent application.

18. Therefore, at least for these reasons given above, the subject-matter of claim 1 of auxiliary request 2, and thus, auxiliary request 2, contravenes Article 123(2) EPC.

Auxiliary requests 3 to 7
Article 123(2) EPC

19. The reasons given above for the main request and auxiliary requests 1 and 2 are relevant for, and apply also to, auxiliary requests 3 to 7.

19.1 The feature "a lysate with a concentration of about 4 M guanidinium" is present in step a) of claim 1 of all these auxiliary requests. As stated above in the context of auxiliary request 2, this feature does not comply with Article 123(2) EPC. Thus, at least for this feature alone and the reasons given in point 16 *et seq.* above, none of auxiliary requests 3 to 7 complies with Article 123(2) EPC.

19.2 Contrary to the method of claim 48 of the patent application and the method disclosed on page 9, lines 21 to 28 of the patent application, the method of claim 1 of auxiliary requests 4 and 6 does not comprise the intermediate step of "capturing the small RNA molecules", after eluting the RNA and the small RNA molecules from the solid support comprising silica and

before using or characterizing the RNA molecules. Therefore, the comments made in points 13 and 14 above in the context of auxiliary request 2 apply also to these auxiliary requests 4 and 6.

19.3 Step b) of auxiliary request 3 is identical to the corresponding step in auxiliary request 2 and thus the reasons given in point 17 *et seq*. above are relevant and apply also to auxiliary request 3. Therefore, also for these reasons, auxiliary request 3 does not comply with Article 123(2) EPC.

19.4 In step b) of claim 1 of auxiliary requests 4 and 5, the concentration of alcohol in the lysate/ethanol mixture has the following discrete values "35%, 45%, 50%, 55%, 60%, 65%, or 70%". These values have to be selected among all other specific values given in the generic disclosure of page 6, lines 20 to 25 of the patent application. These values are also disclosed in Example 6 of the patent application (cf. page 29, lines 26 to 28) and not in Example 1 as stated by the appellant. The method disclosed in Example 6 uses several frozen tissues from mice as starting material and, after homogenization with lysis buffer, absolute ethanol is "added to make final concentrations of 35, 40, 45, 50, 55, 60, 65, and 70% ethanol". There is however no mention in the method disclosed in this example of an extraction of the small RNA molecules from the lysate with an extraction solution comprising phenol prior to the addition of the absolute ethanol. Therefore, in particular the reasons mentioned in point 17.2 above apply to auxiliary requests 4 and 5 as well.

19.5 The amendment introduced into step b) of claim 1 of auxiliary requests 6 and 7, namely that the

concentration of alcohol in the lysate/ethanol mixture is 55%, might have a basis in the generic disclosure on page 6, lines 27 and 28 of the patent application, wherein it is stated that "[i]n specific embodiments, the amount of alcohol solution added to the lysate gives it an alcohol concentration of 55%" and thereafter stating that "[e]thanol is specifically contemplated for use in aspects of the invention" (cf. page 6, lines 29 and 30), as well as in several of the examples of the patent application, such as in Example 1 (cf. page 25, lines 4 and 5), Example 7 (cf. page 30, lines 22 and 23), and Example 8 (cf. page 31, lines 10 and 11). However, as stated above, the combination of this feature with other features present in claim 1 of these auxiliary requests, as well as these other features *per se*, does not have a basis in the patent application. Therefore, auxiliary requests 6 and 7 contravene Article 123(2) EPC.

20. In view of all these considerations, none of auxiliary requests 3 to 7 complies with Article 123(2) EPC.

Conclusion

21. Since neither the main request nor any of auxiliary requests 1 to 7 complies with Article 123(2) EPC, appellant's request to set aside the decision under appeal cannot be granted and therefore the appeal must be dismissed.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

C. Rodríguez Rodríguez T. Sommerfeld

Decision electronically authenticated