CLAIMS

1. A method for isolating RNA, including small RNA having a length of 200nt or less, from a sample, comprising the following steps:

   a) providing a composition comprising RNA and a chaotropic agent;

   b) adding alcohol;

   c) incubating the mixture for at least 2 min;

   d) adding additional alcohol to the mixture to adjust the overall alcohol concentration in the mixture to ≥ 50%;

   e) binding RNA contained in the mixture to a nucleic acid binding solid phase, wherein after step e), RNA including small RNA is bound to the solid phase;

   f) optionally washing the bound RNA;

   g) optionally eluting RNA from the solid phase.

2. The method according to claim 1, **wherein** in step c) a proteolytic digest using a proteolytic enzyme is performed and wherein preferably, the proteolytic enzyme is added prior to step b).

3. The method according to claim 1 or 2, **wherein**

   i) the amount of alcohol added in step b) corresponds to approx. 40% to 80%, preferably 55% to 65%, of the overall alcohol concentration that is added for binding in step e); and/or

   ii) an amount of alcohol is added in step b) so that the resulting mixture comprises the alcohol in a concentration that lies in a range selected from 25% (v/v) to 45% (v/v), preferably 30% to 40% (v/v), more preferred 32.5% to 38% (v/v).

4. The method according to one or more of claims 1 to 3, **wherein** in step c):

   i) incubation occurs at a temperature between 15°C to 30°C; and/or

   ii) incubation occurs for at least 2.5minutes, at least 3 minutes, at least 5 minutes, at least 7.5 minutes or at least 10 minutes.

5. The method according to one or more of claims 2 to 4, **wherein** the mixture that is incubated in step c) comprises:

- RNA;
- a chaotropic salt in a concentration selected from 1 to 5M, 1.5 to 4.5M, 2 to 4 M and 2.5M to 3.75M;
- a proteolytic enzyme, preferably Proteinase K;
- alcohol in a concentration selected from ≥ 25% (v/v) and ≤ 42.5% (v/v), preferably selected from ≥ 32.5% (v/v) and ≤ 40% (v/v).

6. The method according to one or more of claims 1 to 5, **wherein** the alcohol that is added in step b) and in step d) has one or more of the following characteristics:

i. it is a branched or unbranched aliphatic alcohol with 1 to 5 carbon atoms;
ii. it is selected from methanol, ethanol, propanol, isopropanol and butanol and mixtures thereof;
iii. it is selected from isopropanol and ethanol;
iv. it is ethanol.

7. The method according to one or more of claims 1 to 6, **wherein** the composition provided in step a) has been obtained by performing at least the following steps:

- obtaining an RNA containing biological sample;
- lysing the sample wherein lysis preferably involves the use of at least one chaotropic salt;
- optionally homogenising the lysate;
- optionally clearing the lysate.

8. The method according to one or more of claims 1 to 7, **wherein** the composition provided in step a) has been obtained by performing at least the following steps:

- obtaining an RNA and DNA containing biological sample;
- lysing the sample wherein lysis preferably involves the use of at least one chaotropic salt;
- optionally homogenising the lysate;
- optionally clearing the lysate;
- removing DNA from the lysate.

9. The method according to claim 8, **wherein** DNA is removed from the lysate by performing one or more of the following:

- performing a DNAse digest in the lysate;
- selectively binding DNA to a nucleic acid binding solid phase and separating the bound DNA from the remaining sample, thereby providing a DNA depleted RNA containing composition for step a) of the method according to claim 1.

10. The method according to one or more of claims 1 to 9, **wherein** the nucleic acid binding solid phase is

i. comprised in a column;

or

ii. is provided by particles, preferably magnetic particles;

and wherein preferably the nucleic acid binding solid phase is a silicon containing material such as silica, a polysilicic acid material, a borosilicate, a silicate or an anorganic glass.

11. The method according to one or more of claims 1 to 10, **wherein** in step e) a nucleic acid binding phase comprised in a column is used and after RNA binding an on-column DNAse digest is performed; wherein, preferably, after said DNAse digest the following steps are performed:

- collecting small RNA which might have been released from the nucleic acid binding solid phase during the DNAase digest as flow through;
- contacting said flow through which comprises small RNA mixed with a recovery solution with a nucleic acid binding solid phase for binding the contained small RNA to said nucleic acid binding solid phase.

12. The method according to one or more of claims 1 to 11, **wherein** magnetic particles are used as nucleic acid binding solid phase and wherein said method has one or more of the following features:

i) the magnetic particles are added in one of steps a) to e),
ii) an RNA and DNA containing sample is lysed in the presence of a chaotropic agent thereby providing an RNA containing composition for step a) of claim 1, steps b) to d) are performed and after step e), RNA and DNA, including small RNA, is bound to the magnetic particles, after binding, one or more washing steps and a DNase digest is performed, after performing the DNase digest a chaotropic agent and alcohol is added to rebind RNA that was potentially released during the DNase digest to the magnetic particles, optionally one or

more washing steps are performed and the RNA is eluted from the magnetic particles.

13. The method according to one or more of the preceding claims 1 to 12, **wherein** said method is for the parallel isolation of RNA and DNA, wherein said method comprises the following steps:

- obtaining an RNA and DNA containing biological sample;
- lysing the sample wherein lysis involves the use of at least one chaotropic salt;
- optionally homogenising the lysate;
- optionally clearing the lysate;

wherein the isolation of the DNA comprises the following steps:

a) removing DNA from the lysate by selectively binding DNA to a nucleic acid binding solid phase and separating the bound DNA from the remaining sample, thereby providing a DNA depleted RNA containing composition which comprises a chaotropic salt that can be used in step a) of the RNA isolation;

b) optionally washing the bound DNA;

c) optionally performing a proteolytic digest while the DNA is bound to the nucleic acid binding solid phase;

d) optionally washing the bound DNA;

e) optionally eluting the bound DNA;

and wherein the isolation of RNA comprises the following steps:

a) obtaining the DNA depleted RNA containing composition obtained after step a) of the DNA isolation process, wherein said composition comprises a chaotropic salt;

b) adding alcohol thereby providing a mixture which comprises the alcohol in a concentration of ≥ 25% (v/v) and ≤ 42.5% (v/v);

c) incubating the mixture for at least 2min in the presence of a proteolytic enzyme;

d) adding additional alcohol to the mixture to adjust the overall alcohol concentration in the mixture to ≥ 55%;

e) binding RNA and small RNA contained in the mixture to a nucleic acid binding solid phase;

f) optionally washing the bound RNA;

g) optionally eluting RNA from the solid phase.

14. The method according to claim 13, **wherein** in the DNA isolation a proteolytic digest is performed in step c) while the DNA is bound to the nucleic acid binding solid phase and wherein said proteolytic digest has one or more of the following features:

i) a proteolytic enzyme, preferably a protease, is used and the proteolytic digest occurs in the presence of alcohol and a chaotropic salt;
ii) for performing the proteolytic digest a proteolytic composition is added which comprises a proteolytic enzyme, alcohol and a chaotropic salt;
iii) the proteolytic digest is performed at a temperature from 15°C to 30°C; and/or
iv) the proteolytic digest occurs for a time period selected from 3min to 45min, preferably 5min to 30min.

15. The method according to claim 13 or 14, **wherein** in the RNA isolation a DNase digest is performed after the RNA was bound to the nucleic acid binding solid phase in step e) and wherein small RNA potentially released during the DNase digest is rebound to the solid phase.

16. The method according to one or more of claims 1 to 15, having one or more of the following characteristics:

i) the nucleic acid binding solid phase is a silicon containing material such as silica, a polysilicic acid material, a borosilicate, a silicate or an anorganic glass;
ii) the sample is a cell containing sample;
iii) the sample is a tissue sample; and/or
iv) the sample is a fibrous tissue sample.

What is claimed is:

1. An invention not claimed in any divisional application.