fibre membrane. However, the described nucleic acid loading amounts of the membrane are very low and would therefore require huge membrane areas for the large-scale production (g to kg) of mRNA.

WO2014/152966 discloses a method for purifying *in vitro* transcribed RNA, wherein after RNA *in vitro* transcription the reaction mixture is treated with a protein denaturing agent such as urea and then subjected to tangential flow filtration using a hollow fiber membrane.

There remains a need for further RNA purification methods, and in particular for those that allow cost- and time-efficient purification of RNAs at an industrial scale with high yield and pharmaceutical-grade purity, stability and/or shelf life.

It is thus an object of the present invention to provide further RNA production and purification methods suitable for large scale RNA preparation.

**Summary**

In one aspect, the present invention refers to a method for producing and purifying RNA, comprising the steps of A) providing DNA encoding the RNA; B) *in vitro* transcription of the DNA to yield a solution comprising transcribed RNA; and C) diafiltration and/or concentration and/or purification of the solution comprising transcribed RNA by one or more steps of tangential flow filtration (TFF), wherein the method comprises in step C) the steps: C1) optionally termination of transcription; C2) diafiltration and/or oncentration and/or purification of the solution comprising the transcribed RNA by one or more steps of TFF; C3) purification of the RNA by a further purification method, wherein said further purification method does not comprise a step of phenol/chloroform extraction and/or DNA and/or RNA precipitation, wherein the further purification method is performed by means of reversed phase HPLC; and C4) diafiltration and/or concentration and/or purification of the solution comprising the transcribed RNA obtained after step C3) by one or more steps of TFF, wherein the at least one or more steps of TFF comprises using a TFF membrane cassette. In another aspect, the present invention refers to a method for producing and purifying RNA, comprising the steps of a) providing plasmid DNA encoding the RNA; b) linearization of the plasmid DNA in a linearization reaction; c) optionally termination of the linearization reaction; d) concentration of the linearization reaction comprising linearized plasmid DNA by one step of TFF; e) diafiltration of the linearization reaction comprising linearized plasmid DNA by one step of TFF; f) transcription of the DNA to yield a solution comprising transcribed RNA; g) optionally

termination of transcription; h) diafiltration of the solution comprising the transcribed RNA by one step of TFF; i) purification of the RNA by at least one further purification method, wherein the further purification method is performed by means of reversed phase HPLC; j) concentration of the solution comprising the transcribed RNA obtained after step I) by one step of TFF; and k) diafiltration of the solution comprising the transcribed RNA obtained after step J) by a first and a second step of TFF. The objective is solved by a method for producing and purifying RNA, comprising the steps of

A) providing DNA encoding the RNA;

B) transcription of the DNA to yield a solution comprising transcribed RNA; and

C) conditioning and/or purifying of the solution comprising transcribed RNA by one or more steps of tangential flow filtration (TFF).

**Definitions:**

For the sake of clarity and readability the following definitions are provided. Any technical feature mentioned for these definitions may be read on each and every embodiment of the invention. Additional definitions and explanations may be specifically provided in the context of these embodiments.

Enzyme: Enzymes are catalytically active biomolecules that perform biochemical reactions such as DNA dependent RNA transcription (e.g. RNA polymerases), or

## Claims

1. A method for producing and purifying RNA, comprising the steps of

A) providing DNA encoding the RNA;

B) *in vitro* transcription of the DNA to yield a solution comprising transcribed RNA; and

C) diafiltration and/or concentration and/or purification of the solution comprising transcribed RNA by one or more steps of tangential flow filtration (TFF), wherein the method comprises in step C) the steps:

C1) optionally termination of transcription;

C2) diafiltration and/or concentration and/or purification of the solution comprising the transcribed RNA by one or more steps of TFF;

C3) purification of the RNA by a further purification method, wherein said further purification method does not comprise a step of phenol/chloroform extraction and/or DNA and/or RNA precipitation, wherein the further purification method is performed by means of reversed phase HPLC; and

C4) diafiltration and/or concentration and/or purification of the solution comprising the transcribed RNA obtained after step C3) by one or more steps of TFF,

wherein the at least one or more steps of TFF comprises using a TFF membrane cassette.

2. The method according to claim 1, wherein in step A) plasmid DNA is provided as DNA encoding the RNA and the method comprises subsequently to step A) the steps:

A1) linearization of the plasmid DNA in a linearization reaction;

A2) optionally termination of the linearization reaction; and

A3) diafiltration and/or concentration and/or purification of the linearization reaction comprising linearized plasmid DNA by one or more steps of TFF.

3. The method according to claim 1, wherein step C) comprises at least one diafiltration step using TFF and/or at least one concentration step using TFF.

4. The method according to claim 3, wherein the at least one diafiltration step using TFF in step C) comprises diafiltration with an aqueous salt solution.

5. The method according to claim 4, wherein the aqueous salt solution is a NaCl solution.

6. The method according to claim 3, wherein the at least one diafiltration step using TFF of step C) comprises diafiltration with water.

7.The method according to claim 1, wherein the method does not comprise a step of using a TFF hollow fiber membrane.

8. The method according to claim 1, wherein the at least one or more steps of TFF comprises using a TFF membrane with a molecular weight cutoff of $\leq$ 500 kDa, preferably of $\leq$ 200 kDa and most preferably of $\leq$ 100 kDa.

9. The method according to claim 1, wherein the at least one or more steps of TFF comprises using a TFF membrane comprising at least one of polyethersulfone (PES), modified polyethersulfone (mPES), a cellulose derivative membrane or combinations thereof.

10. The method according to claim 1, wherein the at least one or more steps of TFF comprises using a TFF membrane comprising a cellulose derivative membrane with a molecular weight cutoff of about 100 kDa.

11. The method according to claim 1, wherein all steps of TFF are performed with the same TFF membrane.

12. The method of claim 11, wherein the TFF membrane is a cellulose derivative membrane.

13. A method for producing and purifying RNA, comprising the steps of
a) providing plasmid DNA encoding the RNA;
b) linearization of the plasmid DNA in a linearization reaction;
c) optionally termination of the linearization reaction;
d) concentration of the linearization reaction comprising linearized plasmid DNA by one step of TFF;
e) diafiltration of the linearization reaction comprising linearized plasmid DNA by one step

of TFF;

f) transcription of the DNA to yield a solution comprising transcribed RNA;

g) optionally termination of transcription;

h) diafiltration of the solution comprising the transcribed RNA by one step of TFF;

i) purification of the RNA by at least one further purification method, wherein the further purification method is performed by means of reversed phase HPLC;

j) concentration of the solution comprising the transcribed RNA obtained after step I) by one step of TFF; and

k) diafiltration of the solution comprising the transcribed RNA obtained after step J) by a first and a second step of TFF.