

DEVICE AND METHOD FOR PRODUCING NUCLEIC ACID MOLECULES**FIELD OF THE INVENTION**

5 The present disclosure relates to devices and methods for performing a purification step during compound production or the purification of molecules such as nucleic acids. More in particular, devices and methods are disclosed allowing purification by means of magnetic particles.

10 BACKGROUND

Currently established manufacturing processes for biomolecules, such as nucleic acids implement many separate manufacturing steps. Particularly, the respective manufacturing steps are performed by several different devices. In addition, the
15 manufacturing of nucleic acids requires a large degree of manual handling in a GMP-regulated laboratory executed by well-trained technical staff. As a consequence, current established manufacturing processes are time-consuming, cost-intensive, and require a lot of laboratory space and laboratory equipment.

20 US 2021/0261897 describes a bioreactor for in vitro transcription of DNA. However, the system mainly focuses on executing the biochemical reaction step of the RNA production chain and does not provide a detailed method or device for purifying the resulting product.

25 Magnetic bead separation is a technique that is commonly used at lab scale for the purification of compounds generally in the field of testing or at an early stage of research. In general, magnetic particles are linked to a substance that shows an affinity for the compound that needs to be purified. By applying a magnetic field, the magnetic particles with the bound compound of interest are drawn to the
30 magnet, allowing any remaining liquid to be removed. As such, compounds of interest can be separated and/or purified from their liquid environment.

The methodology of magnetic bead purification is often used in the production of biomolecules such as proteins, peptides, antibodies, or nucleic acids.

35 EP1621890 describes a purification device employing magnetic particles. However, it is limited by its use of a vessel that can only accommodate small volumes for elution.

In WO2016123101, magnetic silica nanomembranes are utilized for nucleic acids extraction. Like EP1621890, this method is also constrained to processing small volumes.

- 5 There is a need for large-scale systems to produce and purify biomolecules such as nucleotides. By preference, such systems are fully or semi-automated, in order to enhance the efficiency of the production process and to mitigate human processing mistakes.

10 **SUMMARY OF THE INVENTION**

In a first aspect, the invention relates to a method of processing a liquid sample comprising one or more biochemical products according to claim 1. The method comprises processing said liquid sample with magnetic particles in a processing
15 container and separation of the magnetic particle bound to said biochemical products from the rest of the liquid sample. Embodiments of the method are shown in any of claims 2 to 13.

The processing container comprises an inner and an outer wall having a distance of
20 80 mm or less between them. Said purification containers forms thus a thin layer between the walls where the liquid sample and the biochemical product are processed.

In specific embodiments, according to claims 8 to 13, the biochemical product is a
25 nucleic acid, such as DNA or RNA.

The method of the invention is also related to a cell-free method of producing biochemical products and further purification of said products.

30 In a second aspect, the invention relates to a system for processing biochemical products, according to claim 14.

Embodiments of said system are shown in any of claims 15 to 20. In specific
embodiments, the processing system is coupled with a production system.

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In a final aspect, the invention relates to a processing container according to claim 21.

DESCRIPTION OF FIGURES

The following description of the figures of specific embodiments of the invention is merely exemplary in nature and is not intended to limit the present teachings, their application or uses. Throughout the drawings, corresponding reference numerals indicate like or corresponding parts and features.

- FIG. 1** shows a top view of a processing or purification container according to an embodiment of the invention.
- 10 **FIG. 2** shows a bottom view of a processing or purification container according to an embodiment of the invention.
- FIG. 3** shows a cross-section of a processing or purification container according to an embodiment of the invention.
- 15 **FIG. 4A** depicts a mixing station and **FIG. 4B** depicts the fitting of a processing or purification container on a raised portion of a mixing station according to embodiments of the invention.
- FIG. 5A** depicts a magnetic separation station and **FIG. 5B** depicts the fitting of a processing or purification container to a raised portion of a magnetic separation station
- 20 **FIG. 6** depicts two magnetic separation stations used with a processing or purification device, according to an embodiment of the invention.
- FIG. 7** depicts a system for the production and purification of nucleic acids according to an embodiment of the invention.
- 25 **FIG. 8A** depicts a support rack that holds adaptors **FIG. 8B** shows a representation of a robotic arm grasping an adaptor according to embodiments of the invention.
- FIG. 9A** depicts a processing or purification container with a nested arrangement according and **FIG. 9B** depicts a magnetic separation station according to embodiments of the invention.
- 30 **FIG. 10** depicts embodiments of the purification container according to embodiments of the invention.
- FIG. 11** depicts arrangement on magnets on the magnetic separation station, according to embodiments of the invention.

DETAILED DESCRIPTION

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Described herein are systems and methods for the production and/or purification of cell-free synthesized biochemical products, such as nucleic acids. Said methods and systems provide for large-scale, (semi-continuous or continuous) production of

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those products and aims to resolve at least some of the problems and disadvantages mentioned above.

Definitions

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Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention.

As used herein, the following terms have the following meanings:

“A”, “an”, and “the” as used herein refers to both singular and plural referents unless the context clearly dictates otherwise. By way of example, “a compartment” refers to one or more than one compartment.

“About” as used herein referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of +/- 20% or less, preferably +/-10% or less, more preferably +/-5% or less, even more preferably +/-1% or less, and still more preferably +/-0.1% or less of and from the specified value, in so far such variations are appropriate to perform in the disclosed invention. However, it is to be understood that the value to which the modifier “about” refers is itself also specifically disclosed.

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“Comprise”, “comprising”, and “comprises” and “comprised of” as used herein are synonymous with “include”, “including”, “includes” or “contain”, “containing”, “contains” and are inclusive or open-ended terms that specifies the presence of what follows e.g. component and do not exclude or preclude the presence of additional, non-recited components, features, element, members, steps, known in the art or disclosed therein.

Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order, unless specified. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

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The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within that range, as well as the recited endpoints.

5 The expression "% by weight", "weight percent", "%wt" or "wt%", here and throughout the description unless otherwise defined, refers to the relative weight of the respective component based on the overall weight of the formulation.

10 Whereas the terms "one or more" or "at least one", such as one or more or at least one member(s) of a group of members, is clear *per se*, by means of further exemplification, the term encompasses *inter alia* a reference to any one of said members, or to any two or more of said members, such as, e.g., any ≥ 3 , ≥ 4 , ≥ 5 , ≥ 6 or ≥ 7 etc. of said members, and up to all said members.

15 The terms "purification container" and "processing container" are interchangeably used herein.

As used herein the term "magnetic particle" and variations thereof is intended to denote a particle with a magnetic, e.g., paramagnetic or superparamagnetic, core coated with at least one material having a surface to which biochemical products can reversibly bind. Suitable magnetic particles can include, for example, carboxyl coated paramagnetic particles, silica-based paramagnetic particles, and the like. In embodiments described herein, the magnetic particles are silica-based magnetic particles wherein said silica-based particles can comprise, in some embodiments, a paramagnetic core coated with siliceous oxide, thus providing a hydrous siliceous oxide adsorptive surface to which nucleic acid can bind (e.g., a surface comprising silanol groups). In other or further embodiments, the magnetic particles can be coated with ligand which interacts with biochemical products or can be surface-modified to produce functionalized surfaces, such as weakly or strongly positively charged, weakly or strongly negatively charged, or hydrophobic surfaces, to name a few. In other embodiments, the magnetic particles are coated with surfaces that interact with proteins. Non-limiting examples of such magnetic particles include Dynabeads protein A/G, coated with a mixture of protein A and protein G, MagneHis Ni-Particles coated with nickel-chelating resin, M-280 Streptavidin Dynabeads, MyOne T1 Streptavidin C1 Dynabeads or PureProteome™ Protein A Magnetic Beads coated with protein A, which can bind to the Fc region of immunoglobulins.

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The term "cell-free synthesized biochemical product" or "biochemical product", as interchangeably used herein refers to any biochemical product that is produced,

synthesized, expressed, transcribed, or translated outside of a living cell. Non-limiting examples of cell-free synthesized biochemically include any nucleic acids such as DNA or RNA, including any known species of RNA, peptide, polypeptide, or proteins such as enzymes, antibodies, antigens or vaccines. The process of
5 producing cell-free biochemicals replicates the natural synthesizing machinery without the confinement of living cells membrane.

Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one
10 of ordinary skill in the art to which this invention belongs. By means of further guidance, definitions for the terms used in the description are included to better appreciate the teaching of the present invention. The terms or definitions used herein are provided solely to aid in the understanding of the invention.

15 Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment" or "in an
20 embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to a person skilled in the art from this disclosure, in one or more embodiments. Furthermore, while some embodiments described herein include
25 some but not other features included in other embodiments, combinations of features of different embodiments are meant to be within the scope of the invention, and form different embodiments, as would be understood by those in the art. For example, in the following claims, any of the claimed embodiments can be used in any combination.

30 **Methods**

Methods of purification of cell-free synthesized biochemical products

In a first aspect, described herein is a method of processing or purifying a liquid
35 sample comprising one or more cell-free synthesized biochemical products. Said processing or purifying comprises combining at least a fraction of said liquid sample with a plurality of magnetic particles to form a reactant mixture in a processing or purifying container, wherein the plurality of magnetic particles are able to bind to at

least a portion of said biochemical products in the liquid sample. A magnetic source is applied to the processing container and the biochemical products bound to the magnetic particles are separated from an unbound fraction of the liquid sample. At least a portion of said unbound fraction is removed and the plurality of magnetic particles bound to the biochemical products are retained. The retained magnetic particles are optionally washed with a washing buffer. The processing container comprises at least an inner wall and an outer wall to form a processing space and wherein a distance between the inner wall and outer wall is 80 mm or less.

The method is (semi)automated and allows for continuous or discontinuous purification of cell-free synthesized biochemical products. Often, when using magnetic particles for purification purposes, these magnetic particles are present in tubes or vessels, carrying a substantial volume of magnetic particles. The inventors have found that by making use of a thin layer of magnetic particles in said purification container, the process of binding to the magnetic particles is more efficient and results in high yields of high-quality purified biochemical products.

In an embodiment, the magnetic particles are present in said purification container in a liquid layer, having a layer thickness of less than 100 mm, 95 mm, 90 mm, 85 mm, 80 mm, 75 mm, 70 mm, 65 mm, 60 mm, 55 mm, 50 mm, 45 mm, 40 mm, 35 mm, 30 mm, 25 mm, 20 mm, 15 mm, 10 mm, 9 mm, 8 mm, 7 mm, 6 mm, 5 mm, 4 mm, 3 mm, 2 mm, or 1 mm.

In another embodiment thickness of the layer is between 3 and 75 mm, 4 and 75 mm, 5 and 75 mm, 6 and 75 mm, 7 and 75 mm, 8 and 75 mm, 9 and 75 mm, 10 and 75 mm, 15 and 75 mm, 20 and 75 mm, 25 and 75 mm, 30 and 75 mm, 35 and 75 mm, 40 and 75 mm, 45 and 75 mm, 50 and 75 mm, 55 and 75 mm, 60 and 75 mm, or 65 and 75 mm and all the ranges and subranges in between.

Alternatively, the thickness of the layer is between 3 and 65 mm, 3 and 60 mm, 3 and 55 mm, 3 and 50 mm, 3 and 45 mm, 3 and 40 mm, 3 and 35 mm, 3 and 30 mm, 3 and 25 mm, 3 and 20 mm, 3 and 15 mm, 3 and 10 mm, 3 and 9 mm, 3 and 8 mm, 3 and 7 mm, 3 and 6 mm, 3 and 5 mm or 3 and 4 mm and all the ranges and subranges in between.

In an embodiment said liquid layer is formed between an outer and an inner wall of a purification container. In an embodiment, such a purification container comprises a hollow central cavity lined by the inner walls. An inner volume is formed between said inner and outer wall, open at the top of said container, allowing a component

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and/or liquid to be added in the inner volume. The inner volume of said purification container is configured to receive the liquid comprising the biochemical product and /or magnetic particles. The hollow central cavity is configured to be fitted on the raised portion of a mixing and/or magnetic separation station.

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In some embodiments, a single processing space is formed between the inner wall and the outer wall of the processing or purification container. In other embodiments, a plurality of processing spaces may be formed between the inner wall and the outer wall of the processing or purification container. In some embodiments at least 2, 3, 10 4, 5, 6, 7, 8, 9, or 10 processing spaces are formed between the inner wall and the outer wall of the processing or purification container. In such embodiments, the plurality of the processing spaces is formed by subdividing inner space between the inner wall and outer wall, with additional vertical walls.

15 In an embodiment, an amount of magnetic particles is added or is present between the outer and inner walls of said purification container. In some embodiments, the liquid sample is added or is present between the outer and inner walls of said purification container. Alternatively, the purification container comprises magnetic particles and a liquid sample is provided to said purification container. In yet another 20 alternative embodiment, the purification container comprises the liquid sample and the magnetic particles are provided to said container. In yet another alternative embodiment, the purification container comprises magnetic particles and additional magnetic particles and/or liquid samples are added to said container.

25 The biochemical products in the purification container are allowed to bind to magnetic particles, after which an unbound liquid fraction of the sample is removed and, optionally said magnetic particles bound to said biochemical products are washed by a washing buffer.

30 The steps comprising sample-magnetic particle binding and consecutive magnetic separation, are hereafter referred as "binding steps". In some embodiments of the method as disclosed herein, binding steps are successively repeated multiple times before proceeding with the next steps. As such, in embodiments, binding steps of the present method can be repeated one or more times before proceeding to 35 washing, preferably 1 to 20 times, more preferably 1 to 10 times. In embodiments, the binding steps are successively repeated up to 20 times, up to 15 times, up to 10 times, up to 9 times, up to 8 times, up to 7 times, up to 6 times, up to 5 times, up to 4 times, up to 3 times, up to 2 times, preferably between 5 to 15 times.

It was surprisingly observed that repeating a plurality of binding steps before proceeding to washing and/or elution, thus omitting several rounds of washing and/or elution in between, increases the yield of the biochemical product purified, reduces the quantities of buffer required during purification which reflects in reduced use of hazardous agent(s) such as chaotropic agent(s) like guanidinium thiocyanate and solvents such as ethanol which are commonly used in practice in high quantities. The method also allows for the reuse of the magnetic particles and reduced consumption of magnetic particles overall. The method as disclosed herein which employs concentration of the molecules of interest on the magnetic particles, makes the purification technology safe, sustainable, and economical.

In embodiments, cumulative repeated binding steps can reduce the total alcohol consumption by 95%, 90%, 85%, 80%, 75%, 70%, 60%, 40%, 30%, 20%, 10%, 5% per unit of nucleic acid compared to the method where single binding step is applied. In embodiments the alcohol can be ethanol.

In embodiments, the reduction in use of alcohol when successive binding steps are applied, can be due to the reduction of alcohol, such as ethanol, used in washing steps of the disclosed method. In embodiments, cumulative repeated binding steps can reduce the total alcohol consumption up to 95%, 90%, 85%, 80%, 75%, 70%, 60%, 40%, 30%, 20%, 10%, 5% per unit of nucleic acid compared to the method where single binding step is applied. In embodiments, the alcohol can be ethanol.

In embodiments, these successive binding steps can be performed either by successively adding magnetic particles and biochemical products at each cycle (hereafter referred as the first option) or by placing all magnetic particles in the purification container in one step and successively biochemical products at each cycle (hereafter referred as second option). When the second option is chosen the magnetic particles added in one step should be in a sufficient concentration to provide enough binding capacity for the total amount of biochemical products added in the following successive cycles. It should be obvious to the skilled person the ratio of magnetic particle concentration to biochemical products in the first cycle of successive binding steps is higher in the second option than in the first option. In embodiments, the magnetic particle concentration added in the first cycle of successive binding repeat is 20 times higher in the second option than the first option, for example, 15 times higher, 10 times higher, 9 times higher, 8 times higher, 7 times higher, 6 times higher, 5 times higher, 4 times higher, 3 times

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higher, 2 times higher in the second option than the first option for the purification of the same amount of biochemical products.

5 In an alternative embodiment, each binding step is followed by a consecutive washing step.

10 In some embodiments of the method, the biochemical products bind to the magnetic particles and subsequently, the magnetic particles are captured or induced to move towards a magnet present in the vicinity of the purification container, thereby causing a separation of the biochemical products bound to said magnetic particles and any remaining sample liquid. It is understood that the separation of magnetic particles from the solution also entails physical separation, as in removing the supernatant from the modified magnetic particles.

15 In some embodiments, a binding buffer is used to mediate the reversible binding between the compound of interest and the magnetic particles. In a further embodiment said binding buffer can comprise a chaotropic agent, alcohol, a PEG, a salt, or a mixture thereof. Said chaotropic agent can be chosen from guanidine salts, such as hydrochloride (GuHCl) and guanidium thiocyanate (GuSCN); lithium salts, 20 such as lithium acetate and lithium perchlorate; or sodium salts such as NaCl and combinations thereof. In an embodiment, said binding buffers are devoid of chaotropic agents.

25 The alcohol can be chosen from isopropanol, ethanol, methanol, butanol, and combinations thereof. In some embodiments, the at least one alcohol can be ethanol which is more suitable for the purpose of production of medicaments when compared to commonly used isopropanol taught in prior art. In an embodiment, said alcohol is present at a concentration of 10% to 50%, from 10% to 40%, from 10% to 30%, from 10% to 20%, from 15% to 20% v/v, including all ranges and 30 subranges therebetween.

In an embodiment, said binding buffer may comprise PEG, either as an alternative to the alcohol or in combination with said alcohol. The concentration of PEG in the binding buffer can range from 10% to 40%, from 20 to 40%, from 20% to 35%, 35 from 20% to 30% or from 25% to 35%, including all ranges and subranges therebetween. In an embodiment, 30% PEG is used. In an embodiment, said PEG used in the binding buffer is chosen from PEG 600, PEG 1000, PEG 2000, PEG 3000,

PEG 4000, PEG 6000, PEG 8000, PEG 10.000, and PEG 20.000. In an embodiment, the PEG used is PEG 8000.

- 5 The at least one salt can be present in the binding buffer in a concentration ranging from 0.1M to 5 M, for example, from 0.1 to 4M, from 0.1M to 3M, from 0.1M to 2M, from 0.1M to 1M, from 0.5 to 1M, from 0.5 to 2M, from 1M to 2M and from 2M and 3M and from 3M to 5M, including all ranges and subranges therebetween. According to various embodiments, the at least one salt can be sodium chloride (NaCl).
- 10 In an embodiment, the binding buffer comprises Tris-HCl, NaCl, EDTA, and ethanol. In other embodiments, said binding buffer does not comprise an alcohol but only Tris-HCl, NaCl and EDTA. Non limiting examples of binding buffer compositions include: 40 mM Tris-HCl, 4.8M NaCl, 4 mM EDTA at a pH of 7.4.
- 15 In embodiments, said binding buffer can have a pH ranging from 5 to 10, such as from 5 to 9, from 5.5 to 8.5, from 6 to 8, or from 6.4 to 7.5 and all ranges and subranges therein between. In embodiments, said binding buffer comprise of at least one alcohol and/or PEG, at least one salt and at least one optional chelating agent such as EDTA.
- 20 In some embodiments, said binding buffer is devoid of toxic chaotropic agents such as guanidine salts (guanidinium thiocyanate or guanidine thiocyanate), iodide, perchlorate and trichloroacetate, preferably guanidine salts.
- 25 The magnetic particles can be present in the binding buffer in a concentration ranging, for instance, from about 0.1 µg/µl to about 60 µg/µl, such as from 0.5 µg to about 60 µg/µl, from about 0.75 µg/µl to about 55 µg/µl, from about 1 µg/µl to about 50 µg/µl, from about 2 µg/µl to about 45 µg/µl, from about 3 µg/µl to about 40 µg/µl, from about 4 µg/µl to about 35 µg/µl, from about 5 µg/µl to about 30
- 30 µg/µl, from about 6 µg/µl to about 25 µg/µl, from about 7 µg/µl to about 20 µg/µl, from about 8 µg/µl to about 15 µg/µl, or from about 9 µg/µl to about 10 µg/µl, including all ranges and subranges therebetween. By way of non-limiting embodiment, the at least one magnetic particle may be chosen from Qbeads and may be present in the binding buffer B1 in a concentration ranging from about 0.5
- 35 µg/µl to about 5 µg/µl. In alternative embodiments, the at least one magnetic particle may be chosen from Grace beads and may be present in the binding buffer B1 in a concentration ranging from about 2 µg/µl to about 60 µg/µl. In yet another

alternative embodiment the magnetic particles may be chosen from GreenMat beads at concentrations ranging between 150 and 300 mg/ml, preferably 225 mg/ml.

In specific embodiments, such as where repeated binding steps are preferred, additional magnetic particles can be added to the binding buffer. The further addition of magnetic beads will increase the final concentration of magnetic beads in the binding buffer. In embodiments, the additional magnetic particles added to the binding buffer can result in the magnetic bead concentration in said binding buffer up to 20 times, for example 15 times, 10 times, 5 times, 2 times more than 60 $\mu\text{g}/\mu\text{l}$, as disclosed above. For example, the final one magnetic particle concentration present in the binding buffer can range from 0.1 $\mu\text{g}/\mu\text{l}$ to 1200 $\mu\text{g}/\mu\text{l}$, for instance, from 1 $\mu\text{g}/\mu\text{l}$ to 1100 $\mu\text{g}/\mu\text{l}$, from 10 $\mu\text{g}/\mu\text{l}$ to 1000 $\mu\text{g}/\mu\text{l}$, from 20 $\mu\text{g}/\mu\text{l}$ to 800 $\mu\text{g}/\mu\text{l}$, from 30 $\mu\text{g}/\mu\text{l}$ to 700 $\mu\text{g}/\mu\text{l}$, from 40 $\mu\text{g}/\mu\text{l}$ to 600 $\mu\text{g}/\mu\text{l}$, 40 $\mu\text{g}/\mu\text{l}$ to 500 $\mu\text{g}/\mu\text{l}$, from 50 $\mu\text{g}/\mu\text{l}$ to 400 $\mu\text{g}/\mu\text{l}$, from 60 $\mu\text{g}/\mu\text{l}$ to 300 $\mu\text{g}/\mu\text{l}$, from 70 $\mu\text{g}/\mu\text{l}$ to 200 $\mu\text{g}/\mu\text{l}$, from 80 $\mu\text{g}/\mu\text{l}$ to 150 $\mu\text{g}/\mu\text{l}$, from 600 $\mu\text{g}/\mu\text{l}$ to 1200 $\mu\text{g}/\mu\text{l}$, from 700 $\mu\text{g}/\mu\text{l}$ to 1200 $\mu\text{g}/\mu\text{l}$, from 800 $\mu\text{g}/\mu\text{l}$ to 1200 $\mu\text{g}/\mu\text{l}$, from 900 $\mu\text{g}/\mu\text{l}$ to 1200 $\mu\text{g}/\mu\text{l}$, from 1000 $\mu\text{g}/\mu\text{l}$ to 1200 $\mu\text{g}/\mu\text{l}$, or 0.1 $\mu\text{g}/\mu\text{l}$ to 1200 $\mu\text{g}/\mu\text{l}$, from 0.1 $\mu\text{g}/\mu\text{l}$ to 1100 $\mu\text{g}/\mu\text{l}$, 0.1 $\mu\text{g}/\mu\text{l}$ to 1000 $\mu\text{g}/\mu\text{l}$, from 0.1 $\mu\text{g}/\mu\text{l}$ to 900 $\mu\text{g}/\mu\text{l}$, from 0.1 $\mu\text{g}/\mu\text{l}$ to 800 $\mu\text{g}/\mu\text{l}$, 0.1 $\mu\text{g}/\mu\text{l}$ to 700 $\mu\text{g}/\mu\text{l}$, from 0.1 $\mu\text{g}/\mu\text{l}$ to 600 $\mu\text{g}/\mu\text{l}$, from 0.1 $\mu\text{g}/\mu\text{l}$ to 500 $\mu\text{g}/\mu\text{l}$, 0.1 $\mu\text{g}/\mu\text{l}$ to 400 $\mu\text{g}/\mu\text{l}$, from 0.1 $\mu\text{g}/\mu\text{l}$ to 300 $\mu\text{g}/\mu\text{l}$, 0.1 $\mu\text{g}/\mu\text{l}$ to 200 $\mu\text{g}/\mu\text{l}$, 0.1 $\mu\text{g}/\mu\text{l}$ to 100 $\mu\text{g}/\mu\text{l}$, 1 $\mu\text{g}/\mu\text{l}$ to 100 $\mu\text{g}/\mu\text{l}$, 10 $\mu\text{g}/\mu\text{l}$ to 100 $\mu\text{g}/\mu\text{l}$, or 50 $\mu\text{g}/\mu\text{l}$ to 100 $\mu\text{g}/\mu\text{l}$ including all ranges and subranges therebetween.

In embodiments of the methods as taught herein, a volumetric ratio between the sample and the binding buffer can range, for example, from 1 :1 to 1 :3, such as from 1 :1 to 1 :1.5, or from 1 :1.5 to about 1 :2.5, including all ranges and subranges therebetween. Incubation time period for the mixed solution comprising the biochemical product of interest, the binding buffer and silica-based magnetic particles can range from 0.1 minute to 30 minutes, from 0.1 minutes to 25 minutes, from 0.1 minutes to 20 min, from 0.1 to 10 minutes, or from 0.1 to 5 minutes, from 0.1 to 2 minutes including all ranges and subranges therebetween.

In specific embodiments, such as where the repeated binding steps are preferred, a volumetric ratio between the further added sample in a repeating step and the binding buffer can range, for example, from 1:1 to 1:3, such as from 1:1 to 1:1.5, or from 1:1.5 to about 1:2.5, including all ranges and subranges therebetween. When successive binding steps are repeated, most of the magnetic beads and most of the sample added in the preceding rounds of binding remain in the purification

container in which the further sample and optionally binding buffer is added. Most of the sample added in the preceding rounds of binding steps is expected to be attached to the magnetic particles. It will be obvious to a skilled person that the ratio of overall sample (including sample added in all rounds of binding steps and the last further added sample) to the binding buffer will be higher than indicated above. A volumetric ratio between the overall sample and the binding buffer can range, for example, from 1:3 to 20:1, such as from 1:3 to 1:2, from 1:2 to 1:1, from 1:3 to 20:1, from 1:3 to 19:1, from 1:3 to 18:1, from 1:3 to 17:1, from 1:3 to 16:1, from 1:3 to 15:1, from 1:3 to 14:1, from 1:3 to 13:1, from 1:3 to 12:1, from 1:3 to 11:1, from 1:3 to 10:1, from 1:3 to 9:1, from 1:3 to 8:1, from 1:3 to 7:1, from 1:3 to 6:1, from 1:3 to 4:1, from 1:3 to 3:1, from 1:3 to 2:1 including all ranges and subranges therebetween.

Without being bound to theory, the salt(s) and alcohol(s) introduced by the binding buffer enhance the ability of biochemical products such as nucleic acid, for example RNA, DNA, or protein to reversibly (e.g., non-covalently) bind to the surface of the magnetic particle, such as a silica surface. The magnetic particles thus modified, e.g., comprising reversibly bound biochemical products, can then be separated from the unbound contaminants such as salts, proteins, enzymes, oligonucleotides, DNA templates, and nucleotide triphosphates (NTPs).

After the separation of the liquid and the modified magnetic particles using a magnet, the modified magnetic particles may be in some embodiments, washed with one or more wash buffers. It will be clear to a skilled person that washing entails both addition and removal of the wash buffer to the modified beads. In each successive washing step, the modified magnetic particles are separated from the wash buffer by magnetic separation before proceeding with the next washing and/or moving to any other following step, like the elution step.

A wash buffer can comprise, for example, at least one alcohol and optionally at least one salt. The modified magnetic particles can be rinsed once or multiple times with the wash buffer, and any additional washing can employ the same or different compositions, concentrations, and/or volumetric amounts. It is preferred that the washing buffer is added to the purification container comprising the modified magnetic particles.

In preferred embodiments, the modified magnetic particles are incubated with the wash buffer and then separated at least 2 to 10 times, 2 to 8 times, 2 to 7 times, 2

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to 6 times, 2 to 5 times, 2 to 4 times, or 2 to 3 times, preferably at least 2 to 5 times. Alternatively, steps (b) to (d) are repeated at least 3 to 10 times, 4 to 10 times, 5 to 10 times, 6 to 10 times, 7 to 10 times, 8 to 10 times, or 9 to 10 times.

- 5 According to various embodiments, the at least one alcohol of the washing buffer can be chosen from isopropanol, methanol, ethanol, butanol, and combinations thereof. According to various embodiments, the at least one salt of the washing buffer can be chosen from ammonium sulfate ((NH₄)₂SO₄), ammonium acetate (NH₄Ac), lithium acetate (LiAc), potassium acetate (KAc), sodium acetate (NaAc),
10 sodium chloride (NaCl), and combinations thereof.

In embodiments of the methods as taught herein, said wash buffer comprises at least one alcohol in a concentration ranging, from 50% to 100% by volume/volume (v/v), from 55% to 95%, from 60% to 85%, or from 60% to 80% by v/v, including
15 all ranges and subranges therebetween. The at least one alcohol in the wash buffer can be the same or different from the one alcohol in the binding buffer. In some embodiments, the alcohol in the binding buffer can be ethanol. In non-limiting embodiments, the wash buffer optionally has at least one salt. The optional salt, if present, in the binding buffer is in a concentration ranging from 0.1 M to 5 M, for
20 example, from 0.3 M to 4 M, from 0.1 M to 3 M, from 0.1 M to 2 M, from 0.1 M to 1 M, and from 1 M to 2 M, including all ranges and subranges therebetween. According to various embodiments, the salt in the wash buffer can be sodium chloride (NaCl). Non-limiting examples of wash buffer composition include ethanol at 80% together with 20% RNAase-free water.

25 In some embodiments, the modified magnetic particles can be washed one or more times with at least one of said wash buffer. For example, said modified magnetic particles may be washed once, twice, or more with the wash buffer with intervals of separation of the modified magnetic particles by using a magnet in between the
30 washes.

After the last binding and/or wash step, the modified magnetic particles, comprising the biochemical product of interest reversibly bound to the surface by multiple purification steps, are substantially free of contaminants such as salts, proteins,
35 enzymes, etc. According to various embodiments, the modified magnetic particles thus produced can then be incubated with one or more elution buffers to release the bound biochemical product of interest and separate it from the magnetic particles.

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It should be clear to the skilled person that in some embodiments, the elution step may be repeated multiple times to increase the yield. For example, the elution is repeated 2 times, 4 times, 5 times, 6 times 7 times, 8 times, 9 times, and 10 times. However, in some embodiments a single elution step is performed.

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According to various embodiments, the elution buffer is a low conductivity solution wherein the conductivity of the buffer ranges from 0.001 to 40 mS/cm, more preferably from 0.01 to 40 mS/cm, from 0.1 to 40 mS/cm, from 0.5 to 40 mS/cm, more preferably from 0.5 to 30 mS/cm, from 0.5 to 20 mS/cm, from 0.5 to 10

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mS/cm, including all ranges and subranges therebetween.

In another or further embodiment, said elution buffer comprises a salt concentration of between 0.01 and 50 mM, more preferably between 0.1 to 40 mM, more preferably between 0.1 and 30 mM, more preferably between 0.1 and 20 mM.

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Possible salts include sodium citrate, sodium chloride, sodium phosphate, potassium chloride, potassium phosphate and combinations thereof.

The pH of the elution buffer can range, for example, from 5 to about 10, such as from 5.5 to about 9, from 6 to 8, or from 6.4 to about 7.5, including all ranges and

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subranges therebetween.

For example, in some cases elution buffer can comprise water such as but not limited to RNase free water; in others water and EDTA, or only Tris, or Tris and EDTA, or Sodium citrate, or phosphate buffer, or Phosphate-buffered saline (PBS). The

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concentration of the sodium citrate, if used as elution buffer can range from 0.5 mM to 10mM, for example from 0.6mM to 5mM, from 1mM to 2mM, including all ranges and subranges therebetween. The pH of the Sodium citrate, if used as elution buffer can range from pH 5.4 to 7.5, from pH 6 to pH 7, from pH 6 to 6pH 6.5, including all ranges and subranges therebetween. According to non-limiting embodiments,

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the elution buffer can comprise water or 10 mM Tris-HCl, 1 mM EDTA, pH 7.4, or 10 mM Tris-HCl, pH 7.4-, or 1-mM citrate Na, pH 6.4.

In an embodiment, said elution buffer is devoid of toxic chaotropic agents such as guanidine salts (guanidinium thiocyanate or guanidine thiocyanate), iodide,

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perchlorate and trichloroacetate, preferably guanidine salts.

In some embodiments of the method as described herein, at least one modified particle incubated with the elution buffer for a period ranging from 30 seconds to

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30 minutes, from 1 minute to 20 minutes, from 1 minute to 10 minutes including all the ranges and subranges therebetween.

5 The free magnetic particles (no longer attached to the biochemical particle) can subsequently be removed from the solution, e.g., separated using a magnet, yielding a purified nucleic acid in solution as the final product. The free magnetic particles may further be reused for further rounds of nucleic acid production.

10 The modified magnetic beads can be incubated with the elution buffer for additional rounds (e.g., once, twice, or more) for optimal elution of the purified nucleic acid. In a further embodiment, the elution buffer comprising the nucleic acid molecules is removed from said sample container and stored in a harvest vessel.

15 In an embodiment of the method as disclosed herein, the magnetic particles and the biochemical products in said processing or purification container are mixed by means of subjecting said processing or purification container to a mechanical motion. In some embodiments, the mechanical motion comprises positioning said purification container on a mixing station.

20 The mixing station allows for the mixing of the magnetic particle with biochemical products and the binding buffer and/or of the modified magnetic particle with the washing or elution buffer.

25 In some embodiments, the mixing is ensured by the rotation of at least one wall of the purification or processing container. In other embodiments the inner wall rotates, driving the mixing of the content of the purification or processing container. In other embodiments the outer wall rotates driving the mixing of the content of the purification or processing container. In yet another embodiment both walls rotate driving the mixing of the content of the purification or processing container. In such an embodiment, the wall of the container that is configured to perform rotations may be a ball bearing, but it is not limited to this.

30 In an embodiment the mechanical motion is intermittent rotation, orbital snaking or vibration, but it will be obvious to the skilled person that any mechanical motion that drives the mixing of the content of the purification or processing container may be used with the method disclosed herein.

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In some embodiments, the entire processing or purification container is subjected to a mechanical motion. In other embodiments portions of the purification or processing container are subjected to the mechanical motion.

5 In a further embodiment of the method, as disclosed herein, the applying the magnetic source step occurs at a magnetic separation station wherein said station comprises one or more magnets and further comprises said processing or purification container comprising said magnetic particles and biochemical product sample. The purification container comprising said magnetic particles and biochemical products is positioned at said magnetic separation station, and said
10 magnetic particles are allowed to bind to said magnet during a predefined magnetization time.

By "binding time" it would be understood the time necessary for the biochemical product to bind to the magnetic particles. By "magnetization time" it would be
15 understood the time necessary for the modified magnetic particles to be attracted and become attached to the magnet.

The magnetic separation station allows for incubating the biochemical product with the magnetic particle for a binding time ranging from 5 seconds to 30 minutes, from
20 30 seconds to 20 minutes, and from 1 minute to 10 minutes including all the ranges and subranges therebetween. The modified magnetic particles are then allowed to bind to the magnet for a predefined magnetization time ranging from 20 seconds to 15 minutes, from 20 seconds to 14 minutes, from 20 seconds to 13 minutes, from 20 seconds to 12 minutes, from 20 seconds to 11 minutes, from 20 seconds to 10
25 minutes, from 20 seconds to 9 minutes, from 20 seconds to 8 minutes, from 20 seconds to 7 minutes, from 20 seconds to 6 minutes, from 20 seconds to 5 minutes, from 20 seconds to 4 minutes, from 20 seconds to 3 minutes, from 20 seconds to 2 minutes, from 20 seconds to 1 minute, from 20 seconds to 50 seconds, from 20 seconds to 40 seconds, or from 20 seconds to 30 seconds, including all the ranges and subranges therebetween.
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Alternatively, the modified magnetic particles are then allowed to bind to the magnet for a predefined magnetization time ranging from 30 seconds to 15 minutes, from
35 40 seconds to 15 minutes, from 50 seconds to 15 minutes, from 1 minute to 15 minutes, from 2 minutes to 15 minutes, from 3 minutes to 15 minutes, from 4 minutes to 15 minutes, from 5 minutes to 15 minutes, from 6 minutes to 15 minutes, from 7 minutes to 15 minutes, from 8 minutes to 15 minutes, from 9 minutes to 15 minutes, from 10 minutes to 15 minutes, from 11 minutes to 15

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minutes, from 12 minutes to 15 minutes, from 13 minutes to 15 minutes, or from 14 minutes to 15 minutes, including all the ranges and subranges therebetween.

5 In embodiments of the method as disclosed herein, said magnetization time of the modified magnetic particles increases with the number of purification steps. In some embodiments, the magnetization time increases with 10 seconds to 11 minutes, 10 seconds to 10 minutes, 10 seconds to 9 minutes, 10 seconds to 8 minutes, 10 seconds to 7 minutes, 10 seconds to 6 minutes, 10 seconds to 5 minutes, 10 seconds to 4 minutes, 10 seconds to 3 minutes, 10 seconds to 2 minutes, 10 seconds to 1 minute, 10 seconds to 50 seconds, 10 seconds to 40 seconds, 10 seconds to 30 second, or 10 seconds to 20 seconds, including all the ranges and subranges therebetween with each purification cycle.

15 Alternatively, the magnetization time increases with 20 seconds to 11 minutes, 30 seconds to 11 minutes, 40 seconds to 11 minutes, 50 seconds to 11 minutes, 1 minute to 11 minutes, 2 minutes to 11 minutes, 3 minutes to 11 minutes, 4 minutes to 11 minutes, 5 minutes to 11 minutes, 6 minutes to 11 minutes, 7 minutes to 11 minutes, 8 minutes to 11 minutes, 9 minutes to 11 minutes, 10 minutes to 11 minutes, including all the ranges and subranges therebetween with each purification cycle.

25 The magnetization time of said modified magnetic particles increases with each purification step, to allow the modified magnetic particles sufficient time to bind to said magnet. Each purification step increases magnetic particle's degree of saturation in bound molecules. The magnetizing properties of the magnetic particles decrease and the binding speed to the magnet slows. To avoid the modified magnetic particles not being captured, the inventors increased the magnetization time of each new purification cycle.

30 In an embodiment of the method as disclosed herein, the purification container is moved between the mixing station and the magnetic separation station by means of a robotic arm, configured to grasp said processing or purification container. In a further embodiment, the same purification container is used for the mixing steps and for the binding steps. Alternatively, different containers are used for mixing and binding and the magnetic particles, biochemical products and buffers are transferred between the purification containers by handling means, such as robotic arms.

35 In an embodiment, and in case of multiple purification rounds with increasing magnetization time of the magnetic particles, there is asynchrony in the operation

of the mixing station, operated for a constant time span each purification cycle and the magnetic separation station where the operation time increases with each cycle. This asynchrony allows for purification in multiple purification containers at the same time. To further compensate for the asynchrony and/or if the mixing/separation stations are occupied, a portion of the purification containers may be held as reserve until free mixing/separation stations become available

In a further embodiment, the magnetic particles, liquid sample, binding buffer, washing buffer and/or elution buffer are added to the purification container by pumps, injectors, or robotic arms.

The method as disclosed herein allows for the purification of biochemical products of interest including biopharmaceutical compounds such as DNA, RNA, modified RNA, proteins, peptides, or polypeptides. Advantageously, said method allows automation of separation and/or purification process while executing the procedures continuously and with high precision without requiring human intervention. The purification protocols can be directly implemented on the devices and/or systems without any process scale-up as the device mimics small-scale manual operations executed in a laboratory.

In an embodiment of the method as disclosed herein the purified biochemical products are DNA or RNA.

In an embodiment, the method as disclosed herein relates to the separation and/or purification of RNA molecules. The term RNA or RNA molecules encompasses long-chain RNA, coding RNA, non-coding RNA, long non-coding RNA, single stranded RNA (ssRNA), double stranded RNA (dsRNA), linear RNA (linRNA), circular RNA (circRNA), messenger RNA (mRNA), self-amplifying mRNA (SAM), Trans amplifying mRNA, RNA oligonucleotides, antisense oligonucleotides, small interfering RNA (siRNA), small hairpin RNA (shRNA), antisense RNA (asRNA), CRISPR/Cas9 guide RNAs, riboswitches, immunostimulating RNA (isRNA), ribozymes, aptamers, ribosomal RNA (rRNA), transfer RNA (tRNA), viral RNA (vRNA), retroviral RNA or replicon RNA, small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), microRNA (miRNA), and a Piwi-interacting RNA (piRNA). In some embodiments, said RNA comprises modified RNA molecules. In some embodiments, the modification of RNA molecule comprises chemical modifications comprising backbone modifications as well as sugar modifications or base modifications. In this context, a modified RNA molecule as defined herein comprises nucleotide analogues/modifications, e.g.

backbone modifications, sugar modifications or base modifications. A backbone modification in connection with the present disclosure is a modification, in which phosphates of the backbone of the nucleotides contained in an RNA molecule are chemically modified. A sugar modification in connection with the present disclosure is a chemical modification of the sugar of the nucleotides of the RNA molecule. Furthermore, a base modification in connection with the present disclosure is a chemical modification of the base moiety of the nucleotides of the RNA molecule. In this context, nucleotide analogues or modifications are selected from nucleotide analogues, which are applicable for transcription and/or translation. In further embodiments, the modified RNA comprises nucleoside modifications selected from 6-aza-cytidine, 2-thio-cytidine, α -thio-cytidine, pseudo-iso-cytidine, 5-aminoallyl-uridine, 5-iodo-uridine, N1-methyl-pseudouridine, 5,6-dihydrouridine, α -thio-uridine, 4-thio-uridine, 6-aza-uridine, 5-hydroxy-uridine, deoxy-thymidine, 5-methyl-uridine, pyrrolo-cytidine, inosine, α -thio-guanosine, 6-methyl-guanosine, 5-methyl-cytidine, 8-oxo-guanosine, 7-deaza-guanosine, N1-methyl-adenosine, 2-amino-6-chloro-purine, N6-methyl-2-amino-purine, pseudo-iso-cytidine, 6-chloro-purine, N6-methyl-adenosine, α -thio-adenosine, 8-azido-adenosine, 7-deaza-adenosine.

In a particularly preferred embodiment, the biochemical products are produced in a plurality of reactions, preferably a plurality of in vitro transcription (IVT) reactions, each reaction resulting in a biochemical product sample. Without wishing to be bound by theory, the reaction conditions that need to be fulfilled in an IVT reaction are the provision of linear DNA that serves as a template that is copied into RNA by a polymerase, dNTPs, an RNA polymerase that incorporates the dNTPs, a magnesium-containing buffer that catalyzes the reaction and a specific temperature of the reaction, typically 37°C. It will be obvious to one skilled in the art that any reaction conditions that are yielding mRNA may be used with the method as described herein.

In some embodiments the method as disclosed herein relates to the downstream purification of cell free obtained biochemical products, only. In other embodiments the method as disclosed herein related to the downstream purification of cell free obtained biochemical products and their upstream production.

Methods of production and purification of cell-free synthesized biochemical products

In some embodiments, the downstream purification method as described above is preceded by a cell-free production of a biochemical product. Said biochemical product may be a nucleic acid, such as DNA or RNA, an antibody, a peptide, polypeptide or protein but is not limited to these.

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In an embodiment, the cell-free reactions produce nucleic acids such as DNA or RNA. Non-limiting examples of DNA production methods include the amplification of a template or de novo DNA synthesis. The plurality of said nucleic acid production reactions may thus be PCR reactions and/or DNA synthesis on a solid support that
10 takes place in said reaction chambers. It will be obvious to the skilled person that in such embodiments, the reaction chambers are fitted in devices that provide the required reaction condition, such as temperature incubation, mixing, addition and removal of reagent buffers, enzyme, nucleotides and the like. Other examples of cell-free reactions include in vitro transcription (IVT) for the production of RNA and
15 any other cell-free method for expressing proteins. Non-limiting examples of cell-free protein production methods include the PURE system, the rabbit reticulocyte lysate translation system, the wheat germ lysate translation or any other in vitro translation system that may or may not use ribosomes, tRNAs, amino acids, and/or energy sources

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In an embodiment, both the purification and downstream processing are independent from one another. In another embodiment, said downstream processing or purification and upstream production are coupled.

25 In an embodiment, a plurality of separate cell-free reactions are performed in a plurality of reaction chambers. In an embodiment, the reagents for the plurality of cell-free reactions are added to each reaction chamber. Said reagents are added by handling means such as robotic arms, pumps or injectors. In some embodiments, the reaction reagents are premixed; in other embodiments, the reagents are added
30 independently; in yet other embodiments some of the reagents are premixed while others are added independently than the premix.

In some embodiments, the reagents are thawed, cooled, heated and/or mixed prior to being added to the reaction chambers. It will be obvious to the skilled person that
35 the reactive active and the reaction conditions depend on the type of biochemical product produced and that these reagents and reaction conditions are adapted accordingly.

In a further embodiment of the method, as disclosed herein, at least a portion of the plurality of biochemical product samples from the plurality of chambers is pooled in a pooling vessel. In an embodiment, at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the plurality of biochemical product samples from the plurality of chambers is pooled in said pooling vessel. Preferably at least 25% of the plurality of biochemical product samples from the plurality of chambers is pooled in said pooling vessel. In an embodiment, all biochemical product samples from the plurality of reaction chambers are pooled in said pooling vessel. The total volume of the pooling vessel is preferably equal to or larger than the sum of the volumes of the individual cell-free reactions in the chambers.

In a further embodiment, the pooling of the plurality of biochemical product samples from the plurality of chambers is performed by means of handling apparatuses such as robotic arms, pumps, or injectors.

Pooling of the plurality of biochemical product samples from the plurality of chambers in a pooling vessel provides for intermediate storage and allows for continuous running of the upstream biochemical product production reactions, such as IVT reactions. At the same time, the pooling vessel ensures a continuous supply of molecules for the downstream purification steps.

Alternatively, the method for production and purification as disclosed herein can function without a pooling vessel. In such an embodiment, the content of the reaction chambers is collected directly into one or more purification containers.

In a further embodiment, the method as disclosed herein relates to the downstream purification of the plurality of biochemical products wherein at least a portion of the pooled biochemical product samples from said pooling vessel is transferred to the processing or purification container and subjected to a processing step such as a purification step. In a preferred embodiment, at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the pooled nucleic acid sample from the pooling vessel is transferred to the purification container. Alternatively, all the volume of the pooled biochemical product sample is transferred to the purification container.

In another embodiment, at least a volume portion of the pooled biochemical product samples from said pooling vessel is transferred to a plurality of purification containers. The volume portion of the pooled biochemical product samples from said

pooling vessel is transferred to at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 purification containers, preferably 4 purification containers.

It is preferred that the transfer of the pooled nucleic acid samples from the pooling vessel to said purification container is performed by means of handling apparatuses
5 such as a robotic arm.

The method as disclosed herein may thus make use of a plurality of purification containers. In a preferred embodiment, at least a purification container is used for purification. In another embodiment, at least a purification container is used for purification and one or more purification containers are held as reserve. In another
10 embodiment, a plurality of purification containers are used for purification and one or more purification containers are held as reserve. The temporary storage of biochemical product samples in reserve purification containers ensures a continuous flow of biochemical products between the upstream reaction steps and the downstream purification steps, independent of the duration of each step.

15 Alternatively, all purification containers are used for said purification and no purification container is in reserve.

In a further embodiment of the method as disclosed herein, a further portion of said pooled biochemical product samples is transferred to the processing or purification container comprising biochemical products bound to magnetic particles
20 and subjecting said portion to a binding step.

After each round of completion of the binding of the modified magnetic particles and removal of the liquid fraction, a new volume portion of the pooled biochemical product samples from the pooling vessel is transferred to the purification container, over the modified beads. Said new volume portion of pooled biochemical product is
25 subjected to a new magnetic purification step, wherein said biochemical products in said volume portion are bound to the modified magnetic particles and separated from the unbound liquid fraction of the sample. Washing with a washing buffer is optional. The steps of transfer and binding of the volume portion of biochemical product is repeated until finally a predetermined volume of biochemical product
30 sample in the pooling vessel has been used.

In a preferred embodiment, the steps of transfer and binding of the volume portion of biochemical product are repeated until the majority, preferably all, of the pooled biochemical product samples in said pooling vessel have been subjected to at least
35 one magnetic purification step. In a preferred embodiment the steps of transfer and binding of the volume portion of biochemical products are repeated until at least between 25% and 100%, 30% and 100%, 40% and 100%, 50% and 100%, 60% and 100%, 70% and 100%, 80% and 100% or 90% and 100%, including all the

ranges and subranges therebetween of pooled biochemical product samples in said pooling vessel have been subjected to at least one magnetic purification step.

5 In an alternative preferred embodiment, the steps of transfer and binding of the volume portion of biochemical products are repeated until at least between 25% and 100%, 25% and 90%, 25% and 80%, 25% and 70%, 25% and 60%, 25% and 50%, 25% and 40% or 25% and 30%, including all the ranges and subranges therebetween of pooled biochemical product samples in said pooling vessel have been subjected to at least one magnetic purification step.

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In yet another preferred embodiment, the steps of transfer and binding of the volume portion of biochemical product are repeated until the majority of pooled biochemical product samples in the pooling vessel is substantially free of contaminants such as salts, proteins, enzymes, etc.

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In the embodiments where the need for a pooling vessel has been bypassed, the content of the purification container, namely a sample portion from the reaction chambers, is subjected to a magnetic purification step, after which a new portion of the content of the reaction chambers is added to the modified magnetic particles.

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The steps of transfer and binding of a portions of biochemical products is repeated until finally a predetermined volume of biochemical product sample in the reaction chambers has been used.

25 In an embodiment of the method, as disclosed herein, the biochemical products of an earlier purification cycle remain bound to said magnetic particles when a subsequent purification cycle is initiated. Consequently, the magnetic particles have an increasing accumulation of bound biochemical products with the passing of each purification cycle.

30 In a preferred embodiment, the magnetization time of the magnetic particles to the one or more magnets increases with the number of the processing or purification cycles. The increase in magnetization time is necessary to compensate for the reduction in magnetic attraction of the modified magnetic particles saturated in biochemical products. In preferred embodiments of the method as disclosed herein, 35 said magnetization time of the modified magnetic particles increases with the number of purification cycles. In some embodiments, the magnetization time increases with 110 seconds to 11 minutes, 10 seconds to 10 minutes, 10 seconds to 9 minutes, 10 seconds to 8 minutes, 10 seconds to 7 minutes, 10 seconds to 6

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minutes, 10 seconds to 5 minutes, 10 seconds to 4 minutes, 10 seconds to 3 minutes, 10 seconds to 2 minutes, 10 seconds to 1 minute, 10 seconds to 50 seconds, 10 seconds to 40 seconds, 10 seconds to 30 second, or 10 seconds to 20 seconds, including all the ranges and subranges therebetween with each purification
5 cycle.

Alternatively, the bonding time increases with 20 seconds to 11 minutes, 30 seconds to 11 minutes, 40 seconds to 11 minutes, 50 seconds to 11 minutes, 1 minute to 11 minutes, 2 minutes to 11 minutes, 3 minutes to 11 minutes, 4 minutes to 11
10 minutes, 5 minutes to 11 minutes, 6 minutes to 11 minutes, 7 minutes to 11 minutes, 8 minutes to 11 minutes, 9 minutes to 11 minutes, or 10 minutes to 11 minutes, including all the ranges and subranges therebetween with each purification cycle. The magnetization time of said modified magnetic particles increases with each purification cycle, to allow the modified magnetic particles sufficient time to
15 bind to said magnet. Each purification cycle increases magnetic particle's degree of saturation in bound molecules. The magnetizing properties of the magnetic particles decrease and the binding speed to the magnet slows. To avoid that the modified magnetic particles are not captured, the inventors increased the magnetization time of each new purification cycle.

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It was surprisingly observed that repeating a plurality of purification cycles, namely binding and optional washing, and omitting the elution in between said cycles, increases the yield of biochemical products obtained, reduces the quantities of buffer required during purification which reflects in reduced use of hazardous agent(s) such
25 as chaotropic agent(s) like guanidinium thiocyanate which is commonly used in practice. The method also allows for the reuse of the magnetic particles. Overall, the method as disclosed herein makes the technology safe and affordable.

It is preferred that the binding and washing buffers are added to and removed from
30 the purification container by means of handling apparatuses such as robotic arms, pumps or injectors.

After the last binding and/or wash step, the modified magnetic particles, comprising the compound of interest reversibly bound to the surface by multiple purification
35 cycles, are substantially free of contaminants such as salts, proteins, enzymes, etc. According to various embodiments, the modified magnetic particles thus produced can then be incubated with one or more elution buffers to release the bound

compound of interest and separate it from the magnetic particles. The elution is performed as described in any of the previous embodiments.

The methods disclosed herein can be used to provide a purified biochemical product.

The methods disclosed herein can, in certain embodiments, provide a relative
5 biochemical product yield between 80% to 100% and give sufficient purity. In
embodiments of the methods as taught herein, the purified biochemical product
integrity is conserved while reducing the use of hazardous or unwanted agent(s)
and making the technology affordable by reducing the cost.

In an embodiment of the method, as disclosed herein, the biochemical products are
10 nucleic acid molecules, preferably RNA or DNA.

In an embodiment, the cell-free reactions that are performed are one or more IVT
reactions, producing RNA such as mRNA. In an embodiment, a plurality of IVT
reactions is performed. Each reaction is performed in a chamber.

15 In a further embodiment, at least a part of said IVT reagents are combined into an
IVT premix. In a further embodiment, IVT premix comprises at least dNTPs,
template DNA, and one or more RNA polymerase buffer components. In an
embodiment, the IVT reagents are stored in a storage unit, wherein the storage unit
20 is in fluid connection with a pump system and wherein said pump system is
configured to provide the desired amount of one or more IVT reagents for preparing
an IVT premix to an IVT premix container. Said storage unit may be cooled to a
temperature below 10°C, preferably 4°C, as previously described.

25 The total volume of the premix is equal or larger than the sum of the volumes of
the individual IVT reactions in said chambers, in an embodiment.

In an embodiment, the IVT premix is heated to a temperature of between 35°C to
55°C before a portion of said premix is provided to a chamber of said plurality of
30 chambers. In an embodiment, said IVT premix is heated to a temperature between
35°C and 55 °C, preferably between 35°C and 50 °C, 35°C and 45°C, 35°C and
40°C, or 35°C and 37°C. before a portion of said premix is provided to a chamber
of said plurality of chambers. Alternatively said IVT premix is heated to a
temperature between 37°C and 55 °C, preferably between 40°C and, 45°C and 55°C
35 or 40 and 55°C before a portion of said premix is provided to a chamber of said
plurality of chambers.

In an embodiment, the IVT premix container is heated at a temperature of between 35°C and 55 °C, preferably between 35°C and 50 °C, 35°C and 45°C, 35°C and 40°C, or 35°C and 37°C. Alternatively, the IVT premix container is heated at a temperature of between 37°C and 55 °C, preferably between 40°C and, 45°C and 55°C or 40 and 55°C. In a preferred embodiment, the premix container is heated at 37°C.

In an embodiment of the method disclosed herein, an RNA polymerase is added to at least one chamber of said plurality of chambers. In other embodiments, a plurality of RNA polymerases is added to the chamber, preferably 2, 3, 4, 5, 6, 7, 8, 9, or 10 RNA polymerases. In a further embodiment, said RNA polymerase is added before or after said chamber received a portion of said IVT premix. Combining the IVT premix with the RNA polymerase triggers the start of the reaction. In some embodiments, it is desired that the premix is heated in the absence of the RNA polymerase and thus the start of the reaction is delayed until the premix and the RNA polymerase are combined in the plurality of chambers. In other embodiments, said RNA polymerase is part of said IVT premix. The RNA polymerase used is as previously described.

In some embodiments of the method, the IVT reagents comprise a capping reagent for co-transcriptional capping. Non limiting examples of co-transcriptional capping reagents include CleanCap (TriLink) or ARCA (CellScript). Alternatively, capping occurs post-transcriptional using any cap analog structure known in the art.

The IVT reaction is terminated by adding at least a DNase in an embodiment of the method. In another embodiment, the IVT reaction is terminated by the addition of EDTA. In yet another embodiment, both a DNase and/or EDTA are added to terminate the IVT reaction. The DNase enzymatically digests the DNA template and thus ends the IVT reaction while the EDTA is a chelating agent that depletes the reaction of the Mg^{2+} ions which also leads to the IVT reaction being terminated.

In an embodiment, the DNase and/or EDTA are added in the plurality of chambers. In another embodiment, said DNase and/or EDTA is added to an intermediate vessel where the sample is transferred, such as a pooling vessel. In a further embodiment, the DNase and/or EDTA is added by means of a pump, injector, or robotic arm.

The method as disclosed herein is preferably used for performing a plurality of IVT reactions in the upstream production steps resulting in a plurality of RNA molecules

processed in the downstream purification steps. Nonetheless, it will be obvious for the skilled person that the method may be used for upstream production of other molecules such as but not limited to DNA, that are purified in the downstream steps.

- 5 Preferably, the method as disclosed herein is used for the production and downstream purifying of mRNA molecules from an IVT process, or for purifying during pre-and/or post-capping. In an embodiment, when the mRNA molecule is co-transcriptional capped, said mRNA is purified after the capping. In another embodiment, when the mRNA is post-transcriptionally capped, the purification step
10 is done prior to capping. In yet another or further embodiment, purification is done post-capping. In yet another embodiment, a purification step is done before capping and a second purification step is done post-capping. The IVT resulting product contains besides the desired mRNA product, an array of reaction by-products such as salts, nucleotides, enzymes, proteins, DNA templates, or dsRNA. These can
15 interfere with the capping process and reduce the transaction efficiency and overall purity of the final product. Enzymatic capping immediately following IVT, without intermediate treatment of the reaction product, produces reduced amounts or approaching 0% capped mRNA molecules. The method disclosed herein is designed to perform mRNA purification with high precision, in an automated manner under
20 GMP-compliant conditions, and is adaptable to perform the purification upstream and/or downstream capping. The system allows for the continuous production of small or medium volumes of the compound of interest.

Devices and systems

25 System for downstream processing

- In a second aspect, a system for processing biochemical products by means of magnetic particles is disclosed, wherein said system comprises one or more processing or purification containers; one or more mixing stations, configured to receive a processing or purification container and comprising a motor unit configured
30 to drive a mechanical motioning of said processing or purification container; and one or more magnetic separation stations comprising one or more magnets, and wherein said system further comprises at least one robotic arm, controlled by a computer processor and configured to grasp said processing or purification container and transfer said processing or purification container between said one or more
35 mixing stations and one or more magnetic separation stations, wherein said processing or purification container comprises a wall structure, defining a central cavity I, wherein said wall structure comprises an outer wall and an inner wall defining a processing space between said inner wall and said outer wall, wherein

said wall structure is configured to receive and retain a liquid sample into said inner space.

- The system as disclosed herein allows for the downstream purification of biochemical products by means of magnetic particles. The downstream purification platform is used for purifying or separating the biochemical products from contaminants, debris, reaction products such as template fragments, nucleotides, enzymes, buffers, reagents, and the like.
- 10 In an embodiment, the purification system, as disclosed herein, comprises one or more mixing stations, configured to receive a purification container and comprising a motor unit able to drive a mechanical motioning of said purification container. The mixing station enables the mixing of the contents found inside the purification container. During the binding step, the magnetic particles and the nucleic acid
- 15 molecules are mixed in the binding buffer to ensure an even distribution of the components suspended in the binding buffer. After the magnetic separation, the modified magnetic particles comprising the nucleic acids are optionally mixed with the washing buffer. At the end of the purification cycle, in the elution step, the modified magnetic particles are mixed with the elution buffer.
- 20 In an embodiment of the mixing station, the mechanical motion is rotation around an axis, shaking or a vibrating motion. The rotation around an axis is clockwise in some embodiments, while in other embodiments it is counterclockwise, or both clockwise and counterclockwise.
- 25 In an embodiment, the mechanical motion of the mixing station is driven by a motor unit or electromagnetic unit. In a preferred embodiment, an electric motor is used. In another embodiment, a plurality of electromagnets is powered in sequence to generate the motion of the mixing station. In another embodiment, the mechanical motioning of the mixing station is driven by a shaker unit. The mechanical motion
- 30 of the mixing station can be activated and deactivated. The purpose of the mechanical motioning includes but is not limited to, homogenization of the mixture formed by the sample and the buffers for separation and/or purification, and/or facilitation of the contact between the magnetic particles and the sample.
- 35 In some embodiments, the mixing is ensured by the rotation of at least one wall of the purification or processing container. In other embodiments the inner wall rotated driving the mixing of the content of the purification or processing container. In other embodiments the outer wall rotates driving the mixing of the content of the

purification or processing container. In yet another embodiment both walls rotate driving the mixing of the content of the purification or processing container. In such an embodiment, the wall of the container that is configured to perform rotations may be a ball bearing, but it is not limited to this. In some embodiments, where the mixing is ensured by the rotation of at least one wall, the mixing may be done without a mixing station. Both the mixing and the binding steps may be performed at the same station. In other embodiments, where the mixing is ensured by the rotation of at least one wall, the mixing may be performed at a mixing station and the magnetic separation at a magnetic separation station.

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In a further embodiment of the system, as disclosed herein, the downstream purification platform comprises one or more magnetic separation stations comprising one or more magnets. The magnetic separation station enables the separation of the magnetic particles in a modified or unmodified state, from the liquid of a previous step. After each binding, washing, and elution step, the magnetic particles are captured by the magnet, separating thus the magnetic particles from the liquid in the purification container.

In an embodiment, the magnet of each magnetic separation station comprises a permanent magnet, a temporary magnet, or an electromagnet, preferably a permanent magnet. When an electromagnet is used, the magnetic field can be quickly changed by controlling the amount of electric current. In another embodiment, the magnetic separation station comprises an array of magnets where each magnet can be a permanent magnet, a temporary magnet, or an electromagnet. In another embodiment, the magnetic separation station is equipped with at least two, three, four, five, six, seven, eight, nine, or ten magnets. In some embodiments, the magnetic separation station comprises a magnetic assembly of permanent and/or electromagnetic magnets. It is preferred that the mixing and/or magnetic separation stations comprise a raised portion configured to receive the central cavity of said processing or purification container. The purification container is fitted on said raised portion of the mixing and/or magnetic separation station and is sufficiently secured to be prevented from falling, tumbling, or sliding from the station during the mixing and/or separation.

In other embodiments, the purification or processing container is fitted at the magnetic separation station and/or mixing station by means of a clipping system. It would be obvious that any other method of fitting a container to a station may be used with the system as disclosed herein. In a preferred embodiment, the raised

portion of the magnetic separation station comprises said one or more magnets lining said raised portion. In a further preferred embodiment, the one or more magnets span the entire raised portion of said magnetic separation station. The purification container fitted on said raised portion of the magnetic separation station
5 is in direct contact with the magnet. This ensures that a strong and uniform magnetic field is applied throughout the purification container.

In some embodiments, the one or more magnets are at the interior of the raised portion and are covered by another material. In other embodiments the one or more
10 magnets are both at the inferior and the exterior of said raised portion. Any material may be used in conjunction with the one or more magnets, such as materials that improve the magnetic field generated by electromagnets.

The magnets may be arranged in any shape in the raised portion of the magnetic separation station; none limiting examples include circular, oval, square,
15 rectangular or any polygonal arrangement, cross-shaped arrangement, arrangement in rows or in a line, X-shaped arrangements and the like. In some embodiments the magnets may be separated and controlled independently. In other embodiments the magnets are controlled together. In yet other embodiments the
20 magnets may be separated in groups and the groups may be controlled individually.

In further embodiments of the system, the magnetic separation station comprises a base comprising one or more magnets positioned at said base. In further embodiments, the base of the magnetic separation station has an inclination. The
25 inclined base of the magnetic separation station causes a level difference of the liquid in the inner space of the purification container fitted thereof to have a plurality of heights. The difference in level of the liquid in said container ensures proper removal of the liquid. In some embodiments, the handling means, such as the robotic arm, are thus configured to remove the liquid from the purification container
30 from the region of said purification container with the highest level of the liquid. The magnets are preferably positioned on the base in the region with the smallest thickness of said base, corresponding to the region of the purification container with the highest level of the liquid. The inventors observed that the positioning of the magnets in the preferred liquid removal zone prevents the magnetic particles from
35 being removed together with said liquid.

In a preferred embodiment, the magnetic separation station comprises magnets lining the raised portion and magnets positioned on the base. Alternatively, the magnetic separation station comprises only magnets lining the raised portion.

The raised portions of the mixing and magnetic separation stations have preferably the same shape and size, to allow the fitting of the same purification container. The raised portion has preferably flat lateral surfaces. However, any shape may be adapted for the raised portions of the mixing and magnetic separation stations.

5 In other embodiments, the raised portion of the magnetic separations and/or the mixing stations, surrounds the exterior of the processing or purification container. In such an embodiment, the magnets are positioned at the exterior of the processing or purification container. In such embodiments, the raised portion is ring-shaped. It would be obvious that the shape of the ring is complementary to the shape of the
10 outer wall of the processing or purification container.

In some embodiments, the magnetic separation station has the raised portion inside the cavity of the processing or purification container while the mixing station has the raised portion surrounding the exterior of the processing or purification container. In yet other embodiments, the magnetic separation station has the raised
15 portion surrounding the exterior of the processing or purification container while the mixing station has the raised portion inside the cavity of the processing or purification container.

In a further embodiment, the purification system comprises at least one purification
20 container. The purification container is configured to hold the biochemical products, the magnetic particles, and the buffers, during the purification cycles and elution.

In a preferred embodiment, the purification container comprises a hollow central cavity lined by a wall structure, wherein said wall structure comprises an outer and an inner wall defining an inner volume between said inner and outer wall, wherein
25 said wall structure is open at the top of said container, allowing a component and/or liquid to be added in the inner volume. The inner volume of said purification container is configured to receive the liquid comprising the biochemical product and /or magnetic particles. The central cavity is configured to fit on the raised portion of the mixing and magnetic separation stations. The space formed between the inner
30 and the outer walls is a thin space that surrounds the central cavity and is configured to receive the content of the purification container, namely the magnetic particles, the liquid sample comprising the biochemical product, and the buffers. The shape and the configuration of the purification container allow for the performing of subsequent binding steps while omitting the elution and/or washing steps in
35 between. The elution and/or washing is only performed once at the end when all binding steps have been completed. This significantly increases the yield of the biochemical product obtained, reduces the time necessary for processing, and reduces the quantities of buffer required during purification which reflects in the

reduced use of hazardous agent(s) such as chaotropic agent(s) like guanidinium thiocyanate or ethanol which are commonly used in practice.

In a preferred embodiment of the system, as disclosed herein, the distance between the outer wall and the inner wall of said processing or purification container is less than 80 mm, preferably between 3 and 75 mm, 4 and 75 mm, 5 and 75 mm, 6 and 75 mm, 7 and 75 mm, 8 and 75 mm, 9 and 75 mm, 10 and 75 mm, 15 and 75 mm, 20 and 75 mm, 25 and 75 mm, 30 and 75 mm, 35 and 75 mm, 40 and 75 mm, 45 and 75 mm, 50 and 75 mm, 55 and 75 mm, 60 and 75 mm, 65 and 75 mm, or 70 and 75 mm and all the ranges and subranges in between.

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Alternatively, the distance between the outer and inner walls of said purification container is between 3 and 70 mm, 3 and 65 mm, 3 and 60 mm, 3 and 55 mm, 3 and 50 mm, 3 and 45 mm, 3 and 40 mm, 3 and 35 mm, 3 and 30 mm, 3 and 25 mm, 3 and 20 mm, 3 and 15 mm, 3 and 10 mm, 3 and 9 mm, 3 and 8 mm, 3 and 7 mm, 3 and 6 mm, 3 and 5 mm or 3 and 4 mm and all the ranges and subranges in between.

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As a result of the small distance between the inner and outer walls, the magnetic particles in the purification container are near the magnet and thus can bind to said magnet in a relatively short time, even when saturated with the biochemical product of interest.

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In some embodiments, a single processing space is formed between the inner wall and the outer wall of the processing or purification container. In other embodiments, a plurality of processing spaces may be formed between the inner wall and the outer wall of the processing or purification container. In some embodiments at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 processing spaces are formed between the inner wall and the outer wall of the processing or purification container. In such embodiments, the plurality of the processing spaces is formed by subdividing inner space between the inner wall and outer wall, with additional vertical walls.

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In another embodiment the purification container comprises an inner processing space and an outer processing space and in between said inner processing space and said outer processing space the central cavity configured to receive the raised portion of the mixing and/or magnetic separation stations. In such a nested arrangement, different processing steps may be performed at the same time in the same container. In other embodiments, the purification container has more than two nested processing spaces. It would be obvious to the skilled person that when

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the processing or purification container has more than two nested processing spaces, the mixing and/or magnetic separation stations have multiple raised portions.

- 5 In an embodiment of the system, as disclosed herein, the container has a bottom wall that has an inclination, preferably at an angle of 10°. Said inclination of the bottom wall is configured to cause the liquid added in the inner volume of the purification container to have a difference in level throughout said purification container. The inclination of the bottom wall of the purification container and the
10 inclination of the base of the magnetic separation station are complementary. The higher levels of the liquid in said container ensure proper removal of the liquid.

In an embodiment, the inner and outer walls of the purification container have the same shape in cross-section, which ensures the formation of a thin layer in the inner
15 volume. The cross-section of the inner and/or outer wall of the purification container may have any shape such as, but not limited to pie wedge-shaped, regular or irregular polygon-shaped, concave polygon-shaped, convex polygon-shaped, trigon-shaped, quadrilateral polygon-shaped, pentagon-shaped, hexagon-shaped, equilateral polygon-shaped, equiangular polygon-shaped, heptagon-shaped,
20 octagon-shaped, nonagon-shaped, decagon-shaped, hendecagon-shaped, dodecagon-shaped, tridecagon-shaped, tetradecagon-shaped, pendedecagon-shaped, hexadecagon-shaped, heptadecagon-shaped, octadecagon-shaped, enneadecagon-shaped, icosagon-shaped, n-gon-shaped, elliptic-shaped, or circular- shaped, preferably hexagon-shaped. A purification container having a
25 polygonal cross-section of the inner wall ensures good contact between the magnet and the purification container and thus an optimal exposure of the magnetic particle to the magnetic field.

In some embodiments, the inner wall of the purification container is polygonal in
30 cross-section, comprises a plurality of regular or irregular facets. In specific embodiments of the container, the facets are square, rectangular, trapezoidal, or mixture thereof. This polygonal configuration enhances the surface area available for interaction between the magnetic particles inside the purification container and the magnetic separation station. In particular, when the inner wall comprises a
35 plurality of trapezoidal facets, there is a notable increase in the surface of interaction compared to inner walls with rectangular or square-shaped facets.

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It is preferred that the inner wall of the purification container has the same or a complementary cross section-shape to the cross-section shape of the raised portion of the mixing and magnetic separation stations. This ensures that the purification containers slide onto the raised portion and have optimal contact with the magnet.

- 5 In an alternative embodiment, the inner and outer walls of the purification container have different shapes in cross-section.

It is also preferred that the raised portion of the mixing and magnetic separation stations comprise a plurality of facets having the same shape or complementary shape to the facets of the inner wall.

In a preferred embodiment, at least a purification container is used for purification. In another embodiment, at least a purification container is used for purification and one or more purification containers are held as reserve. In another embodiment, a plurality of purification containers are used for purification and one or more purification containers are held as reserve. Alternatively, all purification containers are used for said purification and no purification container is held as reserve.

20 In some embodiments, the inner volume of the purification container has a capacity of 10 to 100 mL, 10 to 500 mL, 10 to 1000 mL, 10 to 1500 mL. In preferred embodiments, the inner volume of the purification container has a capacity of at least 10 mL, 12 mL, 15 mL, 20 mL, 25 mL, 30 mL, 35 mL, 40 mL, 45 mL, 50 mL, 55 mL, 60 mL, 65 mL, 70 mL, 75 mL, 80 mL, 85 mL, 90 mL, 95 mL, 100 mL, 125 mL, 150 mL, 200 mL, or 250 mL, preferably at least 12 mL.

The magnetic surface of the purification container, as described here, refers to the combined area of all the facets of the inner wall containing the reaction liquid that make contact with the raised portion of the magnetic station. In some embodiments of the container, as disclosed herein, the ratio between the magnetic surface of the purification container (cm^2) and the volume of the liquid inside the container (cm^3) is between 0.5 and 1 cm^{-1} . In some embodiment said ratio is between 0.5 and 0.95 cm^{-1} , between 0.5 and 0.9 cm^{-1} , between 0.5 and 0.85 cm^{-1} , between 0.5 and 0.8 cm^{-1} , between 0.5 and 0.75 cm^{-1} , between 0.5 and 0.7 cm^{-1} , between 0.5 and 0.65 cm^{-1} , between 0.5 and 0.6 cm^{-1} or between 0.5 and 0.65 cm^{-1} .

Alternatively, said ratio is between 0.55 and 1 cm^{-1} , between 0.6 and 1 cm^{-1} , between 0.65 and 1 cm^{-1} , between 0.7 and 1 cm^{-1} , between 0.75 and 1 cm^{-1} ,

between 0.8 and 1 cm⁻¹, between 0.85 and 1 cm⁻¹, between 0.9 and 1 cm⁻¹, between 0.95 and 1 cm⁻¹.

In yet other alternative embodiments said ratio is between 0.5 and 1 cm⁻¹, between 0.55 and 0.95 cm⁻¹, between 0.6 and 0.9 cm⁻¹, between 0.65 and 0.85 cm⁻¹, or between 0.7 and 0.8 cm⁻¹ such as 0.71 cm⁻¹, 0.72 cm⁻¹, 0.73 cm⁻¹, 0.74 cm⁻¹, 0.75 cm⁻¹, 0.76 cm⁻¹, 0.77 cm⁻¹, 0.78 cm⁻¹, or 0.79 cm⁻¹.

It was observed that a small ratio between the magnetic surface of the purification container and the volume of the liquid inside the container enhances the efficiency of magnetization of magnetic particles to the magnet. This is evidenced by the decreased time required for magnetization and the reduced quantity of magnetic particles needed.

The purification container according to the invention advantageously ensures that the liquid in said container is in close proximity to the magnet of the magnetic separation station, preferably at a distance of less than 2 cm, less than 1.95 cm, less than 1.9 cm, less than 1.85 cm, less than 1.8 cm, less than 1.75 cm, less than 1.7 cm, less than 1.65 cm, less than 1.6 cm, less than 1.55 cm, less than 1.5 cm, less than 1.45 cm, less than 1.4 cm, less than 1.35 cm, less than 1.3 cm, less than 1.25 cm, less than 1.2 cm, less than 1.15 cm, less than 1.1 cm, less than 1 cm, less than 0.95 cm, less than 0.9 cm, less than 0.85 cm, less than 0.8 cm, less than 0.75 cm, less than 0.7 cm, less than 0.65 cm, less than 0.6 cm, less than 0.55 cm, less than 0.5 cm, less than 0.45 cm, less than 0.4 cm, less than 0.35 cm or less than 0.3.

In some embodiments, the material of the purification container comprises a material that is resistant to e.g. cleaning procedures (chemically resistant), extreme temperatures (e.g. denaturation of nucleic acids), extreme pH values (sanitization of the reactor with bases and acids, e.g. with NaOH), mechanical forces (e.g. frictions caused by magnetic particles), and/or corrosion. In additional embodiments, the material of the purification container comprises a material of proper light permeation (transparent, translucent, or opaque) to a corresponding purpose. In some embodiments, the material of the purification container comprises a material of proper gas permeation to a corresponding purpose. In further embodiments, the materials of the purification container should be temperature conductive at working temperatures between 37°C and 65°C (e.g. W/(mK) values of at least 10, preferably at least 15). In some embodiments, the inner surface of

the purification container comprises a surface material that does not release unwanted compounds that may contaminate the end product. In further embodiments, the materials of the purification container and/or the inner surface thereof are PC (polycarbonate), PP (polypropylene), PAI (polyamide-imide) (e.g. Torlon), PI (polyimide) (e.g. Tecasint), PPS (polyphenylsulfide) (e.g. Tecatron), PPSU (polyphenylsulfone) (e.g. Tecason P), PSU (Polysulfone) (e.g. Tecason S), PEI (polyetherimid) (e.g. Tecapei), glass (e.g. borosilicate glass), technical ceramics (e.g. FRIDURIT®), Polyaryletherketone (e.g., Polyetheretherketon (PEEK)), thermoplastics (e.g. DuraForm® Pa or DuraForm® GF), all of which being chemically resistant, pH resistant, and temperature resistant. In additional embodiments, the materials of the purification container comprise a material for a single-use including, but not limited to, polyethylene terephthalate and other polyethylenes, polyvinyl acetate, polyvinyl chloride. In some embodiments, the materials of purification container comprise a material having resistance to sterilization process including steam treatment or ethylene oxide (EtO) exposure/gamma irradiation even before adding any reaction-related reagents. In some embodiments, the materials of the purification container provide protection from light (if needed) for medium contained in the purification container. In some embodiments, the purification container is made of any nonmagnetic or paramagnetic material known in the art.

In a further embodiment, the system comprises handling means configured for dispensing, removing, and/or transferring a component or a liquid.

Said particle component can be a magnetic particle and said liquid can be deionized water, purified water, a buffered solution, a binding buffer, a washing buffer, an elution buffer, a reagent, a liquid sample comprising a biochemical product of interest, or a combination thereof, or a waste product. It should be apparent that said liquid is not limited to these.

In a further embodiment, the handling means are configured to grasp a purification container and able to transfer said purification container between said one or more mixing stations and one or more magnetic separation stations.

The handling means may comprise any apparatuses suitable in the art, such as an injector or a robotic arm, for instance, provided with one or more nozzles, needles, and/or tips. Alternatively, the dispensing by the handling means is done directly via the tubing of the handling means. Any injector, pump, or robotic arm that is known in the art and is capable of dispensing and/or removing a component or a liquid can

be used with the system as disclosed herein. Non-limiting examples of handling means are syringe pumps, vacuum pumps, peristaltic pumps, centrifugal pumps, or a combination thereof.

5 In a preferred embodiment, the robotic arm grasps the purification container by an upper portion and transfers said purification container between said one or more mixing stations and one or more magnetic separation stations. In yet a further embodiment the robotic arm is configured to grab an adapter comprising needles and/or injectors for dispensing, removing, and/or transferring a component or a liquid. Said needles and/or injectors are connected to one or more reagent storage, 10 waste vessels, and/or harvest vessels. In yet a further preferred embodiment, the adapters comprising needles and/or injectors are placed by said robotic arm on racks prior to and after utilization.

15 In an embodiment, the injectors and pumps are fixed dispensers placed in the vicinity of said mixing and/or magnetic separation station. In an embodiment, the robotic arm can move along three separate axes and is able to access the reactive vessels, purification container and/or a harvesting vessel.

20 In an embodiment, at least one robotic arm is used in conjunction with the system as disclosed herein. In another embodiment, one or more injectors are used in conjunction with said system. In yet another embodiment one or more pumps are used in conjunction with said system. In yet another embodiment, a robotic arm and one or more injectors are used in conjunction with said system. In yet another embodiment, a robotic arm and one or more pumps are used in conjunction with 25 said system. In a preferred embodiment, a robotic arm, one or more injectors, and one or more pumps are used in conjunction with said system.

The handling means used in conjunction with the system are connected to one or more reagent storage, waste vessels, and/or harvest vessels. Any reagent storage, waste vessels, and harvest vessels known in the art can be used in conjunction with 30 the system. Non-limiting examples include bags, vials, tubes, bottles, jars, or barrels.

35 In an embodiment, the handling means used in conjunction with the system are controlled by motor units, preferably electric motors, and are configured to perform movements. In some embodiments, said movement is a vertical, horizontal, centrifugal, or a 3D movement. In a preferred embodiment, the handling means are operatively coupled to at least one computer processor for controlling said handling apparatuses.

The system may further comprise a control system arranged and adapted to control the dispensing and/or removing of a component or liquid by said handling means used in conjunction with the system. Based on the input information, such as sample ID and sensor data, and predefined algorithms, the control system regulates the performance of the handling means.

In an embodiment, the system, as disclosed herein further comprises at least a computer processor. In another embodiment, the computer processor is operatively coupled with the robotic arm and is configured to control the handling means such as the robotic arm.

In yet another embodiment, the computer processor is operatively coupled to the mixing and/or magnetic separation stations. Preferably, the computer processor comprises instructions to calculate and control a binding time of magnetic particles and the sample comprising the biochemical products and a magnetization time of the modified magnetic particles and the one or more magnets of the magnetic separation station. The computer processor calculates and controls the increasing magnetization time of the magnetic particles, and further takes into consideration the asynchrony in the operation of the mixing station and the magnetic separation station. To further compensate for the asynchrony and/or if the mixing/separation stations are occupied, the computer processor controls when the purification containers may be held as reserve or introduced on available mixing/separation stations.

In yet another embodiment, the computer processor is configured to control the transfer of the purification vessel between the mixing station and the magnetic separation station.

In some embodiments, a single computer processor is coupled to multiple devices and/or apparatuses of the system and is configured to control multiple actions. In other embodiments, each device and/or apparatus of the system is controlled by an individual computer processor. In an embodiment, the system comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 computer processors.

In some embodiments, the mixing station and the magnetic separation station are operated by the same computer processor while in other embodiments the mixing station and the magnetic separation station are operated by different computer processors.

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In a preferred embodiment, the downstream purification system is a stand-alone system capable to function independently. In such an embodiment the system may be arranged in a cabinet or box. The system can, however, function coupled to another system, being other downstream purification systems or upstream systems, such as biochemical product production systems.

System for upstream production and downstream processing

The current invention relates also to a system comprised of at least one upstream production system coupled with at least one downstream purification system

10 Said system comprises:

- a plurality of reaction chambers for producing said biochemical products in a plurality of reactions;
- a pooling vessel for receiving the reactions from the plurality of chambers;
- a downstream purification platform, allowing downstream purification of said biochemical products by means of magnetic particles, wherein said platform comprises one or more mixing stations, configured to receive a purification container and comprising a motor unit able to drive a mechanical motioning of said purification container; and one or more magnetic separation station comprising one or more magnets, and wherein said purification platform further comprises at least one robotic arm, controlled by a computer processor and configured to grasp a purification container and able to transfer said purification container between said one or more mixing stations and one or more magnetic separation stations.

25 Advantageously, the system as disclosed herein allows automation of biochemical product production and downstream purification while executing the procedures continuously and with high precision without requiring human intervention. The production protocols can be directly implemented on the system as disclosed herein without any process scale-up as the device mimics small-scale manual operations executed in a laboratory.

Reaction chambers

The system as disclosed herein comprises a plurality of reaction chambers for the performance of cell free reactions such as an IVT reaction.

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In some embodiments, the reaction chambers are housed in a cartridge. In a further embodiment, the cartridge comprises between 2 and 48 chambers.

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In some embodiments, the cartridge comprises at least 2 chambers, 3 chambers, 4 chambers, 5 chambers, 6 chambers, 7 chambers, 8 chambers, 9 chambers, 10 chambers, 11 chambers, 12 chambers, 13 chambers, 14 chambers, 15 chambers, 16 chambers, 17 chambers, 18 chambers, 19 chambers, 20 chambers, 21 chambers, 22 chambers, 23 chambers, 24 chambers, 25 chambers, 26 chambers, 27 chambers, 28 chambers, 29 chambers, 30 chambers, 31 chambers, 32 chambers, 33 chambers, 34 chambers, 35 chambers, 36 chambers, 37 chambers, 38 chambers, 39 chambers, 40 chambers, 41 chambers, 42 chambers, 43 chambers, 44 chambers, 45 chambers, 46 chambers, 47 chambers, 48 chambers, 49 chambers, or 50 chambers. In some embodiments, the cartridge comprises at least about 60 chambers, about 70 chambers, about 80 chambers, about 90 chambers, about 100 chambers, about 110 chambers, about 120 chambers, about 130 chambers, about 140 chambers, about 150 chambers, about 160 chambers, about 170 chambers, about 180 chambers, about 190 chambers, or about 200 chambers.

In an embodiment, the system comprises 1 cartridge, 2 cartridges, 3 cartridges, 4 cartridges, 5 cartridges, 6 cartridges, 7 cartridges, 8 cartridges, 9 cartridges, or 10 cartridges. In another embodiment, said reaction unit comprises between 1 and 10 cartridges, between 1 and 20 cartridges, between 1 and 30 cartridges or between 1 and 40 cartridges, 1 and 50 cartridges, 1 and 60 cartridges, 1 and 70 cartridges, 1 and 80 cartridges, 1 and 90 cartridges, or 1 and 100 cartridges for nucleic acid production.

In an embodiment, the chamber comprises at least three flat polygonal surfaces. In another embodiment, the chamber comprises one, two, three, four, five, six, seven, or eight flat polygonal surfaces, preferably six flat polygonal surfaces. Advantageously, the design of the chamber allows for it to be equipped with a sensor and/or a probe.

In an embodiment, the cross-section of the chamber is polygonal, such as trigonal, tetragonal, pentagonal, or hexagonal. In a preferred embodiment, the cross-section of the chamber is hexagonal. A plurality of hexagonal chambers, eg combined in a cartridge, may create a honey-comb shaped structure. Without wishing to be bound to theory, the hexagonal shape is known in geometry to best fill a plane with equal size units without leaving out unused space. Moreover, hexagonal packing also minimizes the perimeter for a given area because of its 120-degree angles, and thus the hexagonal shape of the chamber ensures less use of raw material.

In an embodiment, the open end of the chamber is configured to receive a removable lid for at least partially closing said open end. The lid prevents unwanted components from entering said chamber (for example, RNases, microbial contamination or other degrading compounds or organisms) and shields the content of said chamber from the outer environment. In an embodiment, said lid also prevents evaporation of the content of said chamber. In some embodiments, the chamber comprises the lid to limit exchange with the environment. In some embodiments, the lid of the chamber is removable. In some embodiments, the lid of the chamber is not removable. Alternatively, the chamber is uncovered.

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In some embodiments, the lid can prevent excessive water evaporation and loss of other critical volatile components. In some embodiments, the lid can prevent oxidation of the reagents or any components. In some embodiments, the lid can provide with protection from light (if needed). In some embodiments, the lid prevents contamination from any other potential chemical compound.

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In some embodiments, the lid comprises at least one opening for filling, draining, and sampling. In some embodiments, the at least one opening is positioned on the top of the lid.

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In some embodiments, the bottom of the chamber is rounded. In other embodiments, the bottom of the chamber is flat or pointed.

In some embodiments, the chamber is removable from the cartridge. In some embodiments, the chamber is not removable from the cartridge.

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In an embodiment, the chambers comprise a volume of about 0.1 mL to 500 mL.

In some embodiments, the chamber comprises a volume of at least about 0.1 ml, about 0.3 ml, about 0.5 ml, about 1 ml, about 1.5 ml, about 2 ml, about 2.5 ml, about 3 ml, about 4 ml, about 5 ml, about 6 ml, about 7 ml, about 8 ml, about 9 ml, about 10 ml, about 12 ml, about 15 ml, about 17 ml, about 20 ml, about 25 ml, about 30 ml, about 35 ml, about 40 ml, about 45 ml, about 50 ml, about 55 ml, about 60 ml, about 65 ml, about 70 ml, about 75 ml, about 80 ml, about 85 ml, about 90 ml, about 95 ml, or about 100 ml. In some embodiments, the chamber comprises a volume of not more than about 100 ml, not more than about 95 ml, not more than about 90 ml, not more than about 85 ml, not more than about 80 ml, not more than about 75 ml, not more than about 70 ml, not more than about

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65 ml, not more than about 60 ml, not more than about 55 ml, not more than about 50 ml, not more than about 45 ml, not more than about 40 ml, not more than about 35 ml, not more than about 30 ml, not more than about 25 ml, not more than about 20 ml, not more than about 15 ml, not more than about 10 ml, not more than about 9 ml, not more than about 8 ml, not more than about 7 ml, not more than about 6 ml, not more than about 5 ml, not more than about 4 ml, not more than about 3 ml, not more than about 2 ml, not more than about 1 ml, not more than about 0.5 ml, not more than about 0.3 ml, or not more than about 0.1 ml. In some embodiments, the chamber comprises a volume of between about 1 ml to about 100 ml, between about 10 ml to 90 ml, between about 15 ml to about 80 ml, between about 20 ml to about 70 ml, between about 25 ml to about 60 ml or between about 30 ml to about 50 ml.

In some embodiments, the chamber comprises a volume of at least about 150 ml, about 200 ml, about 250 ml, about 300 ml, about 350 ml, about 400 ml, about 450 ml, about 500 ml, about 550 ml, about 600 ml, about 650 ml, about 700 ml, about 750 ml, about 800 ml, about 850 ml, about 900 ml, about 950 ml, about 1000 ml, about 2000 ml, about 3000 ml, about 4000 ml, about 5000 ml, about 6000 ml, about 7000 ml, about 8000 ml, about 9000 ml, about 10000 ml, about 15000 ml, about 20000 ml, about 25000 ml, about 30000 ml, about 35000 ml, about 40000 ml, about 45000 ml, or about 50000 ml. In some embodiments, the chamber comprises a volume of not more than about 50000 ml, not more than about 45000 ml, not more than about 40000 ml, not more than about 35000 ml, not more than about 30000 ml, not more than about 25000 ml, not more than about 20000 ml, not more than about 15000 ml, not more than about 10000 ml, not more than about 9000 ml, not more than about 8000 ml, not more than about 7000 ml, not more than about 6000 ml, not more than about 5000 ml, not more than about 4000 ml, not more than about 3000 ml, not more than about 2000 ml, not more than about 1000 ml, not more than about 950 ml, not more than about 900 ml, not more than about 850 ml, not more than about 800 ml, not more than about 750 ml, not more than about 700 ml, not more than about 650 ml, not more than about 600 ml, not more than about 550 ml, not more than about 500 ml, not more than about 450 ml, not more than about 400 ml, not more than about 350 ml, not more than about 300 ml, not more than about 250 ml, not more than about 200 ml, not more than about 150 ml. In some embodiments, the chamber comprises a volume of between about 150 ml to about 50000 ml, between about 200 ml to 45000 ml, between about 250 ml to about 40000 ml, between about 300 ml to about 35000 ml, between about 350 ml to about 30000 ml, between about 400 ml to about 25000 ml, between

about 450 ml to about 20000 ml, between about 500 ml to about 15000 ml, between about 550 ml to about 10000 ml, between about 600 ml to about 9000 ml, between about 650 ml to 8000 ml, between about 700 ml to about 7000 ml, between about 750 ml to about 6000 ml, between about 800 ml to about 5000 ml, between about 850 ml to about 4000 ml, between about 900 ml to about 3000 ml, between about 950 ml to about 2000 ml or between about 1000 ml to about 1500 ml.

The chambers are used for the production of biochemical products such as nucleic acid molecules, peptides, polypeptides or proteins. It is preferred that said nucleic acid molecule is RNA, more preferably mRNA. Alternatively, said nucleic acid molecules are DNA.

In an embodiment of the system, said system comprises an IVT unit for RNA molecules production.

The IVT unit comprises a plurality of chambers, as disclosed in previous embodiments, wherein each of the plurality of chambers is configured to receive one or more IVT reagents and to execute an IVT reaction.

The IVT unit is to be understood as that part of said system that accommodates an IVT reaction starting from a DNA template, by means of a DNA dependent RNA polymerase. In some embodiments, the DNA dependent RNA polymerases comprise at least one of a T7 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase, an RNA polymerase I, an RNA polymerase II, an RNA polymerase III, an RNA polymerase IV, an RNA polymerase V, and a single subunit RNA polymerase. The DNA template for RNA in vitro RNA transcription may be obtained by cloning of a nucleic acid, in particular cDNA corresponding to the respective RNA to be in vitro transcribed and introducing it into an appropriate vector for RNA in vitro transcription, for example in plasmid circular plasmid DNA. The cDNA may be obtained by reverse transcription of mRNA or chemical synthesis. Moreover, the DNA template for in vitro RNA synthesis may also be obtained by gene synthesis.

In some embodiments, the DNA template relates to a DNA molecule comprising a nucleic acid sequence encoding the RNA sequence. The template DNA is used as a template for RNA in vitro transcription to produce the RNA encoded by the template DNA. Therefore, the template DNA comprises all elements necessary for RNA in vitro transcription, particularly a promoter element for binding of a DNA dependent RNA polymerase as e.g., T3, T7 and SP6 RNA polymerases 5' of the DNA sequence

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encoding the target RNA sequence. The poly(A) tail can be either encoded into the DNA template or added enzymatically to RNA in a separate step after in vitro transcription. In some embodiments, the template DNA comprises primer binding sites 5' and/or 3' of the DNA sequence encoding the target RNA sequence to determine the identity of the DNA sequence encoding the target RNA sequence e.g., by PCR or DNA sequencing. In some embodiments, the DNA template comprises a DNA vector, such as a plasmid DNA, which comprises a nucleic acid sequence encoding the RNA sequence. In some embodiments, the DNA template comprises a linear or a circular DNA molecule.

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In some embodiments, the IVT unit comprises at least one handling apparatus configured for dispensing and/or removing a reagent, a reagent mixture, or a liquid in said chambers. Without being limitative the liquid dispensed and/or removed can be deionized water, purified water, a buffer solution, a cleaning solution, a reagent, an enzyme, a DNA template, a transcription buffer, a solution comprising nucleotide triphosphates (NTPs), an Rnase inhibitor, or an RNA polymerase, or a combination thereof, or a waste product. Non-limitative examples of buffers are magnesium acetate, HEPES, Tris, or Tris HCl. In a preferred embodiment, the liquid dispensed and/or removed is an IVT premix comprising water, RNA polymerase buffer, linearized DNA template, and dNTPs. In a further preferred embodiment, the IVT premix comprises water, T7 polymerase buffer, linearized DNA template, and dNTPs.

The handling apparatuses of the IVT unit can be an injector or a robotic arm provided with one or more nozzles, needles, and/or tips. Any injectors and robotic arms known in the art and capable of dispensing and/or removing a component or liquid can be used with the system as disclosed herein. Non-limiting examples of handling apparatuses are syringe pumps, vacuum pumps, peristaltic pumps, centrifugal pumps, or a combination thereof. In some embodiments, the pipettes, micropipettes, needles, or tips are removable and/or single use. In other embodiments, the pipettes, micropipettes, needles, or tips are not removable.

Alternatively, the dispensing and/or removing of components is done by the handling apparatuses directly via the tubing they are provided with.

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In an embodiment, the IVT unit as disclosed herein comprises a robotic arm. In another embodiment, the IVT unit comprises one or more injectors. In yet another embodiment the IVT unit comprises one or more pumps. In yet another

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embodiment, the IVT unit comprises a robotic arm and one or more injectors. In yet another embodiment, the IVT unit comprises a robotic arm and one or more pumps. In a preferred embodiment, the IVT unit comprises a robotic arm, one or more injectors, and one or more pumps.

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The handling apparatuses of the IVT unit are configured to perform movements. In some embodiments of the IVT unit, said movement is a vertical, horizontal, centrifugal, or a 3D movement. In an embodiment, said handling apparatuses are controlled by motor units, preferably electric motors.

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In some embodiments, the IVT unit comprises a control system arranged and adapted to control the dispensing and/or removing of a reagent, reagent mixture or liquid by said handling apparatuses. Based on the input information, such as sensor data and predefined algorithms, the control system regulates the performance of the handling apparatuses.

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In an embodiment of the IVT unit, the handling apparatuses are adapted to dispense an amount of an IVT premix and/or one or more enzymes in said chambers.

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In an embodiment, the system as disclosed herein comprises heating apparatuses for heating the IVT premix container. In an embodiment, the IVT premix container is heated at a temperature of between 35°C and 55 °C, preferably between 35°C and 50 °C, 35°C and 45°C, 35°C and 40°C, or 35°C and 37°C. Alternatively, the IVT premix container is heated at a temperature of between 37°C and 55 °C, preferably between 40°C and, 45°C and 55°C or 40 and 55°C. In a preferred embodiment, the premix container is heated at 37°C.

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In an alternative embodiment of the system as disclosed herein, the nucleic acid produced is DNA. In such embodiments, the system comprises devices that provide the required reaction condition, such as temperature incubation, mixing, addition and removal of reagent buffers, enzyme, nucleotides, and the like, for PCR reactions and/or DNA synthesis on a solid support.

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In yet another embodiment, the system is used for the production of peptides, polypeptides or proteins. In such embodiments, the system comprises devices that provide the required reaction condition, such as temperature incubation, mixing, addition and removal of reagent buffers, enzyme, tRNAs, aminoacyl-tRNA synthetases, initiation, elongation and termination factors, energy sources (ATP,

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GTP), energy regenerating systems such as creatine phosphate, creatine phosphokinase, phosphoenol pyruvate or pyruvate kinase, co-factors such as Mg^{2+} , K^{+} , and/or starting materials such as DNA or RNA for PCR in vitro protein translation.

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In an embodiment, the system comprises a storage unit for storing one or more reagents, said storage unit can be cooled to a temperature below $10^{\circ}C$. In an embodiment, one reagent, two reagents, three reagents, four reagents, five reagents, six reagents, seven reagents, eight reagents, nine reagents, or ten reagents, preferably four reagents are stored in said storage unit. The reagents can be selected from water, T7 reaction buffer, linearized DNA template, and NTPs but are not limited to these.

In some embodiments, the storage unit can be cooled below $10^{\circ}C$, $9^{\circ}C$, $8^{\circ}C$, $7^{\circ}C$, $6^{\circ}C$, $5^{\circ}C$, $4^{\circ}C$, $3^{\circ}C$ or $1^{\circ}C$, preferably the cooled storage is at $4^{\circ}C$.

The storage unit is in fluid connection with a pump system, such as a peristaltic pump or a syringe pump. In additional embodiments, the pump comprises a vacuum pump, a peristaltic pump, a centrifugal pump, or combination thereof.

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In an embodiment, said fluid connection is via at least one tubing. In some embodiments the tubing comprises, and is preferably made of, a material selected from the group consisting of polyethylene (PE), nylon, urethane, copper, stainless steel, aluminum, polyvinyl chloride (PVC), polypropylene (PP), polyurethane (PU), vinyl, polyvinylidene fluoride (PVDF), fiberglass, glass, rubber, and combinations thereof. In some embodiments, the tubing is chemical resistant.

In an embodiment, the pump system is configured to provide a desired amount of one or more reagents for preparing an IVT premix to an IVT premix container.

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Pooling vessel

The system comprises in preferred embodiments a pooling vessel for receiving the reactions from the plurality of chambers. In some embodiments, the pooling vessel is in fluid communication with the chambers. In other embodiments, the pooling vessel is not in fluid communication with the chamber. In some embodiments, the pooling vessel is configured to store the biochemical products produced in the reaction chambers. In some embodiments, the pooling vessel is configured to detect contamination of the biochemical products. In some embodiments, the pooling

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vessel contains means to perform certain chemical, physical or mechanical treatment of the biochemical products and/or the undesired residuals – for example, binding of the product, partial elimination of residuals, or adjustment of pH value.

- 5 In an embodiment, the pooling vessel has a volume equal to, preferably higher than the volume of a reaction chamber. In a preferred embodiment, the pooling vessel has a volume equal to or higher than the sum of volumes of the reaction chambers of a cartridge. In an alternative embodiment, the pooling vessel has a volume equal to or higher than the sum of volumes of multiple cartridges, preferably of 2, 3, 4,
10 5, 6, 7, 8, 9 or 10 cartridges.

In some embodiments, the pooling vessel comprises a volume of 100 to 1000 mL, more preferably from 250 to 500 mL, more preferably from 250 to 350 mL, and more preferably from 300 to 350 mL, and all ranges and subranges in between. The
15 pooling vessel comprises preferably a volume of around 300 ml.

The pooling vessel may have any shape or size and may be manufactured from any suitable material known in the art, such as any glass or plastic materials, as disclosed in a previous embodiment for the purification container.

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It would be, however, obvious for the skilled person that the system for production and purification as disclosed herein can function without a pooling vessel. In such an embodiment, the content of the reaction chambers is collected directly into a purification container. In such an embodiment the system can be operated in both
25 a continuous and non-continuous manner.

Downstream purification platform

The production and purification system as disclosed herein may further comprises a
30 downstream purification platform, for the purification of the biochemical products produced in the plurality of reaction chambers and pooled in the pooling vessel. The downstream purification platform coupled to the production platform and the pooling vessel, is similar to the independent system for biochemical products purification described in previous embodiments. However, the downstream purification platform
35 is coupled to the biochemical product production unit and the pooling vessel in such a way that it allows for continuous and automated production and purification of biochemical products while mimicking lab-based methods. The downstream purification platform coupled to the production means and the pooling vessel allows

for the successive binding of small portions of biochemical products to magnetic particles, while executing the washing and/or elution at the end. The specific configuration of the purification container that allows for a thin liquid layer, together with the coupling of the downstream purification platform to a pooling vessel and upstream production platform allows for a continuous manner of production and purification molecules which is more efficient, and provides for high yields.

The downstream purification platform of the system as disclosed herein may comprise one or more mixing stations, configured to receive a purification container and comprising a motor unit able to drive a mechanical motioning of said purification container; and one or more magnetic separation station comprising one or more magnets, and wherein said purification platform further comprises at least one robotic arm, controlled by a computer processor and configured to grasp a purification container and able to transfer said purification container between said one or more mixing stations and one or more magnetic separation stations.

In a preferred embodiment, the purification container comprises a hollow central cavity lined by a wall structure, wherein said wall structure comprises an outer and an inner wall defining an inner volume between said inner and outer wall, wherein said wall structure is open at the top of said container, allowing a component and/or liquid to be added in the inner volume. The purification container has thus all the characteristics of the purification containers of the above described embodiments.

In an embodiment, the content of the plurality of the reaction chambers equals or exceeds the content of said purification container. In a preferred embodiment, the content of the reaction chambers of a cartridge is equal to or exceeds the content of said purification container. In an alternative embodiment, the purification container has a content equal to or higher than the sum of the content of multiple cartridges, preferably of 2, 3, 4, 5, 6, 7, 8, 9 or 10 cartridges.

The mixing and magnetic separation station of the downstream purification platform coupled to a biochemical product production unit and/or pooling vessel have all the characteristics of the purification containers of the above described embodiments

35 **Handling means**

In a further embodiment, the system comprises handling means for transferring the content from the reaction chambers to the pooling vessel and to the purification container(s) and the purified biochemical product to the harvesting vessel. In a

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preferred embodiment, the handling means are configured for dispensing, removing and/or transferring a component or a liquid. In a further embodiment the handling means are configured to grasp a purification container and able to transfer said purification container between said one or more mixing stations and one or more magnetic separation stations.

Said particle component can be a magnetic particle and said liquid can be deionized water, purified water, a buffered solution, a washing buffer, an elution buffer, a reagent, or a combination thereof, or a waste product. It should be apparent that said liquid is not limited to these.

The handling means may comprise any apparatuses suitable in the art, such as an injector or a robotic arm, for instance provided with one or more nozzles, needles, and/or tips. Alternatively, the dispensing by the handling means is done directly via the tubing of the handling means. Any injector, pump, or robotic arm that is known in the art and is capable of dispensing and/or removing a component or a liquid can be used with the system as disclosed herein. Non-limiting examples of handling means are syringe pumps, vacuum pumps, peristaltic pumps, centrifugal pumps, or a combination thereof.

In an embodiment, the robotic arms grasp the purification container by an upper portion and transfers said purification container between said one or more mixing stations and one or more magnetic separation stations. In yet a further embodiment the robotic arm is configured to grab an adapter comprising needles and/or injectors for dispensing, removing and/or transferring a component or a liquid. Said needles and/or injectors are connected to one or more reagent storage, waste vessels, and/or harvest vessels. In yet a further embodiment, the adapters comprising needles and/or injectors are placed on racks prior and after utilization by said robotic arm.

In an embodiment, the injectors and pumps are fixed dispensers placed in the vicinity of said reaction chamber or mixing or magnetic separation station. In an embodiment, the robotic arm can move along three separate axes and is able to access the reaction chambers, pooling vessel, purification container and harvesting vessel.

In an embodiment, at least one robotic arm is used in conjunction with the system as disclosed herein. In another embodiment, one or more injectors are used in conjunction with said system. In yet another embodiment one or more pumps are

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used in conjunction with said system. In yet another embodiment, a robotic arm and one or more injectors are used in conjunction with said system. In yet another embodiment, a robotic arm and one or more pumps are used in conjunction with said system. In a preferred embodiment, a robotic arm, one or more injectors, and one or more pumps are used in conjunction with said system.

The handling means used in conjunction with the system are connected to one or more reagent storage, waste vessels, and/or harvest vessels. Any reagent storage, waste vessels, and harvest vessels known in the art can be used in conjunction with the system. Non-limiting examples include bags, vials, tubes, bottles, jars, or barrels.

In an embodiment, the handling means used in conjunction with the system are controlled by motor units, preferably electric motors, and are configured to perform movements. In some embodiments, said movement is a vertical, horizontal, centrifugal, or a 3D movement. In a preferred embodiment, the handling means are operatively coupled to at least one computer processor for controlling said handling apparatuses.

The system may further comprise a control system arranged and adapted to control the dispensing and/or removing of a component or liquid by said handling means used in conjunction with the system. Based on the input information, such as sample ID and sensor data, and predefined algorithms, the control system regulates the performance of the handling means.

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Computer processors

In an embodiment, the system as disclosed herein further comprises at least one computer processor. In another embodiment, the computer processor is operatively coupled with the robotic arm and is configured to control said robotic arm.

In yet another embodiment, the computer processor is operatively coupled to the downstream purification platform. Preferably, the computer processor comprises instructions to calculate and control a binding time of magnetic particles and volume portion. The computer processor may calculate and control the increasing magnetization time of the magnetic particles, and further take into consideration the asynchrony in the operation of the mixing station and the magnetic separation station. To further compensate for the (a)synchrony and/or if the mixing/separation stations are occupied, the computer processor controls when the purification

containers may be held as reserve or introduced on available mixing/separation stations.

5 The computer processor, in an embodiment, comprises instructions to calculate and/or to control the amount of the volume portion of the pooling vessel to be transferred to the purification container and the transfer thereof.

10 The computer processor may be configured in some embodiments to control the pooling of the samples from the plurality of chambers in the pooling vessel. In yet another embodiment, the computer processor is configured to control the transfer of the purification vessel between the mixing station and the magnetic separation station.

15 In some embodiments, a single computer processor is coupled to multiple devices and/or apparatuses of the system and is configured to control multiple actions. In other embodiments, each device and/or apparatus of the system is controlled by an individual computer processor. In an embodiment, the system comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 computer processors.

20 In an embodiment, the biochemical product production device, and the downstream processing platform are operatively coupled to the same computer processor. In another embodiment, the biochemical product production device and the downstream processing platform are operatively coupled to different computer processors. In yet another embodiment the plurality of reaction chambers and the handling apparatuses of the biochemical product production device are operatively
25 coupled to different computer processors. In yet another embodiment the plurality of reaction chambers and the handling apparatuses of the biochemical product production device, are operatively coupled to the same computer processors. In yet another embodiment the downstream purification platform and the handling apparatuses of said platform are operatively coupled to different computer
30 processors. In yet another embodiment the downstream purification platform and the handling apparatuses of said platform are operatively coupled to the same computer processors. In yet another embodiment all handling apparatuses are coupled to the same computer processor.

35 In some embodiments, the mixing station and the magnetic separation station are operated by the same computer processor while in other embodiments the mixing station and the magnetic separation station are operated by different computer processors.

Cabinets

It is preferred that the system as disclosed herein is arranged in a cabinet, preferably with a unit for laminar flow generation. In a further embodiment, said system comprises a storage unit for storing ingredients, wherein said storage unit
5 is positioned outside or inside said cabinet.

In an embodiment, the cabinet is designed to allow the provision of filtered, sterile air to be circulated within the units. Air filtering means may include for instance a HVAC system with HEPA filters.

10 The housing of the cabinet may be made of any material suitable in the art such as metal alloy, metal, or plastic. In one embodiment, a cabinet is made from a material comprising aluminum or stainless steel. In a specifically preferred embodiment, said cabinet is made of a material comprising stainless steel.

Preferably, the system and the cabinet are designed and operated that they only
15 require limited handling of the operator. This is to avoid contamination and disturbance of the process conditions. If irregularities are observed, the operator can manipulate the process via one or more control devices present inside or outside the cabinet. These control devices control (parts of) the process taking place in the cabinet. The cabinet may be coupled to one or more control devices that are
20 configured to perform multivariate analysis, automatically control the operation of the processes, and optionally, communicate with components remotely (using, for example, network protocols) in order to control operation in the unit(s).

Each cabinet is preferably mobile and provided with transportation means. Transportation means can include any means suitable in the art, both manually
25 and/or electronically controlled, and include but are not limited to wheels, tracks or rolls.

Purification or processing container

In a third aspect, the invention relates to a purification container comprising a
30 (hollow) central cavity lined by a wall structure, wherein said wall structure comprises an outer and an inner wall defining an inner volume between said inner and outer wall, wherein said wall structure is open at the top of said container, allowing a component and/or liquid to be added in the inner volume. The purification container has a shape, form, and volume and is made of the materials as described
35 in any of the previous embodiments.

The processing or purification container is preferably open at the top or is configured to open when a liquid or a component is added or removed from the container. In

an embodiment the processing or purification container is completely open at the top. In another embodiment, said processing or purification has an aperture or hole at the top. In yet another embodiment the processing container is not open at the top but has a lid that is removable, slidable, openable or the like.

- 5 In a preferred embodiment of the purification container, as disclosed herein, the distance between the outer and inner wall is between 3 and 75 mm, 4 and 75 mm, 5 and 75 mm, 6 and 75 mm, 7 and 75 mm, 8 and 75 mm, 9 and 75 mm, 10 and 75 mm, 15 and 75 mm, 20 and 75 mm, 25 and 75 mm, 30 and 75 mm, 35 and 75 mm, 40 and 75 mm, 45 and 75 mm, 50 and 75 mm, 55 and 75 mm, 60 and 75 mm, 65 and 75 mm, or between 70 and 75 mm and all the ranges and subranges
10 in between.

- Alternatively, the distance between the outer and inner walls of said purification container is between 3 and 70 mm, 3 and 65 mm, 3 and 60 mm, 3 and 55 mm, 3
15 and 50 mm, 3 and 45 mm, 3 and 40 mm, 3 and 35 mm, 3 and 30 mm, 3 and 25 mm, 3 and 20 mm, 3 and 15 mm, 3 and 10 mm, 3 and 9 mm, 3 and 8 mm, 3 and 7 mm, 3 and 6 mm, 3 and 5 mm or 3 and 4 mm and all the ranges and subranges in between.

- 20 The shape and the configuration of the purification container allow for the performing of multiple cycles of purification, namely binding and liquid removal, while omitting the elution and/or washing steps in between. The elution is only performed at the end, when at least a portion of the content of the pooling vessel has been subjected to at least one purification cycle. This significantly increases the
25 yield of nucleic acid molecules obtained, reduces time necessary for processing, and reduces the quantities of buffer required during purification which reflects in reduced use of hazardous agent(s) such as ethanol which is commonly used in practice. As a result of the small distance between the inner and outer walls, the magnetic particles in the purification container are near the magnet and thus can bind to said
30 magnet in a relatively short time, even when saturated with the biochemical product of interest.

The invention is summarized below in some preferred embodiments

- 35 1. A method of purifying a liquid sample comprising one or more nucleic acid molecules, wherein said purifying comprises the removal or the exchange of at least a fraction of said liquid sample and wherein said purifying comprises a magnetic purification step, wherein said nucleic acid molecules are bound to magnetic

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particles during a binding step in a purification container, after which an unbound liquid fraction of the sample is removed and, optionally, said magnetic particles bound to said nucleic acids are washed by a washing buffer, wherein the magnetic particles are present in said purification container in a liquid layer, having a layer
5 thickness of less than 75 mm.

2. The method according to embodiment 1, wherein the layer thickness is between 3 and 70 mm.
3. The method according to embodiment 1 or 2, wherein said liquid layer is formed between an outer and an inner wall of said purification container.
- 10 4. The method according to any of embodiments 1 to 3, wherein an amount of magnetic particles is added or is present between the outer and inner wall of said purification container.
5. The method according to any of the previous embodiments, wherein the magnetic particles and nucleic acid molecules in said purification container are
15 mixed by means of subjecting said purification container to a mechanical motion, preferably by positioning said purification container on a mixing station.
6. The method according to any of the previous embodiments, wherein the magnetic binding step occurs at a magnetic separation station comprising one or more magnets, wherein said purification container comprising said magnetic
20 particles and nucleic acid sample is positioned at said magnetic separation station, and wherein said magnetic particles are attracted to said magnet during a predefined magnetization time.
7. The method according to any of the previous embodiments, wherein the purification container is moved between the mixing station and the magnetic
25 separation station by means of a robotic arm, configured to grasp said purification container.
8. The method according to any of the previous embodiments, wherein said nucleic acid molecules are DNA or RNA.
9. The method according to embodiment 8, wherein said nucleic acid molecules
30 are produced in a plurality of nucleic acid reactions, preferably a plurality of in vitro transcription reactions.
10. The method according to embodiment 9, wherein at least a portion of the plurality of nucleic acid molecules produced are pooled in a pooling vessel.
11. The method according to embodiment 10, wherein at least a volume portion
35 of the pooled nucleic acid samples from said pooling vessel is transferred to the purification container and subjected to a purification step.
12. The method according to embodiment 10, wherein a further volume portion of said pooled nucleic acid samples is transferred to the purification container

comprising nucleic acids bound to magnetic particles and subjecting said portion to a binding step.

13. The method according to embodiment 12, wherein the magnetization time of the magnetic particles to the magnet increases with the number of purification cycles.

14. A system for performing purification of nucleic acid molecules by means of magnetic particles, wherein said system comprises one or more purification containers; one or more mixing stations, configured to receive a purification container and comprising a motor unit able to drive a mechanical motioning of said purification container; and one or more magnetic separation stations comprising one or more magnets, and wherein said system further comprises at least one robotic arm, controlled by a computer processor and configured to grasp said purification container and transfer said purification container between said one or more mixing stations and one or more magnetic separation stations, wherein said purification container comprises a hollow central cavity defined by a wall structure, wherein said wall structure comprises an outer and an inner wall defining an inner volume between said inner and outer wall, wherein said wall structure is open at the top of said container, allowing a component and/or liquid to be added in the inner volume.

15. The system according to embodiment 14, wherein the distance between the outer and inner walls of said purification container is between 3 and 70 mm

16. The system according to any of embodiments 14 or 15 wherein, the purification container comprises a tilted bottom configured to cause the liquid added in the inner volume of the purification container to have a plurality of heights throughout said purification container.

17. The system according to any of the embodiments 13 to 15, wherein the one or more mixing and/or magnetic stations comprise a raised portion configured to receive the hollow central cavity of said purification container.

18. The system according to embodiment 16, wherein the raised portion of the magnetic separation station comprises one or more magnets lining said raised portion.

19. The system according to any of the embodiments 13 to 17, wherein the magnetic separation station comprises a base comprising one or more magnets positioned at said base.

20. The system according to embodiment 13 wherein, said computer processor comprises instructions to calculate and control a magnetization time of magnetic particles and magnet.

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21. A purification container comprising a hollow central cavity defined by a wall structure, wherein said wall structure comprises an outer and an inner wall defining an inner volume between said inner and outer wall, wherein said wall structure is open at the top of said container, allowing a component and/or liquid to be added
5 in the inner volume.

EXAMPLES

Example 1 RNA production and purification

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IVT reactions were performed in a plurality of chambers. The content of 10 reaction chambers, consisting of 20 ml IVT product were pooled in a pooling vessel where EDTA and dNase were added. The IVT resulting mixture in the pooling vessel comprised a volume of 250 ml with a mRNA concentration of 7-9 mg/ml.

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A volume portion of this 250 ml of IVT mixture was added in the processing container comprising 3.5–4 g of silica-coated magnetic beads and subjected to mixing-binding steps. The process was repeated until the entire volume of 250 ml IVT mixture was used from the pooling vessel. The entire purification cycle of the pooling vessel took
20 100 minutes and produced 1559 mg purified mRNA.

The purification cycle can be repeated continuously as long as the IVT reactor performs IVT reaction and purifies 935.40 mg RNA/h.

- 25 Example 2 Ratio between the magnetic surface of the purification container in contact with the reaction liquid and the volume of the reaction liquid

The magnetic surface of a purification container according to an embodiment of the invention was determined and the ratio between said surface and the volume of
30 liquid inside the container during two operations, namely elution and binding, were calculated (Table 1).

The same ratio was calculated for the surface area of other containers known in the art and their maximum volume capacity. The containers were a standard Eppendorf
35 ® tube of 1500 µl, a standard Falcon ® tube of 50 ml and a 200 ml bottle.

The container according to the invention has a ratio between the magnetic surface of the purification container and the volume of the liquid inside the container of

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between 0.7 and 0.8 (cm⁻¹) which is smaller than said ratio for the other containers used in the art (Table 1) and which increases the efficiency of the magnetization of the magnetic particles to the magnet.

5 In addition, the container according to the invention ensures that the liquid inside the purification container is at a maximum distance from the magnet of the magnetic station of 0.9 cm, which ensures strong magnetization and low time necessary for said magnetization. Using the same volume in prior art containers, the liquid inside the station is at a higher distance from the magnetic station.

10 Table 1.

Purification container according to an embodiment of the invention used for:	Surface of the purification container (cm ²)	Volume of the liquid inside the container (cm ³)	Ratio between surface and volume (cm ⁻¹)	Max. distance to the magnet of the magnetic separation station (cm)
Elution	137.28	194.56	0.71	0.9
Binding	38.16	48.64	0.78	0.9
Standard lab equipment				
Eppendorf tube	2.88	1.5	1.92	0.87
Falcon tube	67.38	48.64	1.38	1.22
Bottle	276.46	194.56	1.42	4

DESCRIPTION OF FIGURES

15 The present invention will now be further exemplified with reference to the following examples. The present invention is in no way limited to the given examples or to the embodiments presented in the figures.

20 FIG.1 is a top view of a processing or purification container, according to an embodiment of the purification system. The container (1) comprises a wall structure composed of an outer wall (3) and an inner wall (2) defining a processing space (4). Said wall structure is open at the top of said container, allowing a component and/or liquid to be added in the inner space. The liquid may be a liquid sample and/or a buffer while the component may be a magnetic particle. The distance between the

outer and the inner wall (8) is in some embodiments less than 75 mm. The magnetic particles are thus present in said purification container in a thin layer.

5 In the embodiment of FIG. 1, the container comprises a knob (5) that has a shape adapted to be grasped by the robotic arm. It will be obvious to the skilled person, that other adaptations may be applied to said purification container in order to make it suitable to be transported/moved/grasped by a robotic arm.

10 The container (1) has a hollow central cavity (6) open at the bottom of said container, lined by the inner wall (2), as depicted in the embodiment of FIG. 2. The processing space (4) between the wall structure is closed at the bottom and has a bottom wall (9). In some embodiments, the inner wall (2) of the container may have an indentation (7). This indentation has an orientation purpose when said purification container is fit on the raised portion of the mixing (11) and/or magnetic separation
15 (17) stations.

As depicted in FIG. 3, the container (1) has an inclination of the bottom wall, configured to cause the liquid added in the processing space (4) to have a difference in level throughout said purification container (10', 10"). The inner wall of said
20 purification container has in the embodiment of FIG. 3 uneven heights (2', 2") which causes the inclination of the bottom wall. The inclination of the bottom wall (9) of the purification container and the inclination of the base of the magnetic separation station causes the liquid in said purification container to have differences in level (10', 10"). The removal of the liquid is done preferably where the liquid sample has
25 maximum level (10') and is preferably avoided where the liquid sample has minimum level (10").

As shown in FIG. 4A, the mixing station (11) comprises a base (12) having a raised portion (13). The base is positioned on a motor unit that drives the motioning of the
30 mixing station. The processing or purification container (1) is fit on the raised portion (13) of the mixing station as shown in FIG. 4B. In some embodiments, the raised portion of the mixing station (13) has an indentation (15) for the correct positioning of said purification container.

35 As shown in FIG. 5A, the magnetic separation station (16) comprises a base (17) on which a raised portion (20) is placed. In a preferred embodiment, the raised portion is lined with a plurality of magnets (18). The raised portion is provided with a plurality of screw holes (19). After the magnets (18) are inserted in the raised

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portion, they are kept in place by a lid (31) fixed in place with screws (32), as shown in FIG. 6. The purification container (1) is fit on the raised portion (20) of the magnetic separation station as shown in FIG. 5B. In some embodiments, the raised portion of the mixing station (16) has an indentation (21) for the correct positioning
5 of said purification container.

As depicted in FIG. 6, in some embodiments the magnetic separation station comprises a base (17) comprising one or more magnets (33) positioned at said base. The base (17) of the magnetic separation station has an inclination. The
10 inclined base of the magnetic separation station causes the liquid in the purification container fit thereof to have level differences. The handling means, such as the robotic arm, are thus configured to preferably remove the liquid from the purification container from the region of said purification container with the highest height of the liquid. The magnets are preferably positioned on the base in the region
15 corresponding to the region of the purification container with the highest height of the liquid.

FIG. 7 shows a system (22) for the production and purification of nucleic acid molecules comprising a plurality of reaction chamber (23). In some embodiments, said reaction chambers are IVT reaction chambers. The system (22) also comprises
20 a pooling vessel (24) where the content of the plurality of reaction chambers is pooled. The system further comprises a mixing station (11), one or more magnetic separation stations (16) and one or more purification containers (1) held as reserve. In addition, the system comprises one or more robotic arms (25) configured to move
25 the purification container and to add/or remove a component or liquid in said purification container. In other embodiments the system may comprise other handling means such as pumps or injectors and or reagent storage containers, temperature control means and the like.

FIG. 8A depicts a support rack that holds adaptors (27) fitted with needles (28) for liquid introduction or suction. The adaptors (27) are connected by tubes (29) to waste or reactive vessels (30). The adaptors have a shape adapted to be grasped
30 by the robotic arm (Figure 8B) .

35 In some embodiments, the processing or purification container (1) may comprise multiple processing spaces (4). As depicted in FIG. 9 said processing or purification container with a nested arrangement comprises an inner processing space (4') and an outer processing space (4'') and in between said inner processing space and said

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outer processing space the central cavity configured to receive the raised portion of the mixing and/or magnetic separation stations (16).

5 In some embodiments, the raised portion of the magnetic separations and/or the mixing stations (16), surrounds the exterior of the processing or purification container (1). In the embodiment of FIG. 9B, the magnets (18) are positioned at the exterior of the processing or purification container. In such embodiments, the raised portion is ring-shaped. It would be obvious that the shape of the ring is complementary to the shape of the outer wall of the processing or purification
10 container.

In some embodiments, the mixing of the liquid sample and the magnetic particles inside the processing or purification container is ensured by the rotation of at least one wall of the purification or processing container. In other embodiments the inner
15 wall (2) rotates driving the mixing of the content of the purification or processing container (FIG. 10C). In other embodiments the outer wall (3) rotates driving the mixing of the content of the purification or processing container (FIG. 10B). In yet another embodiment both walls (2,3) rotate driving the mixing of the content of the purification or processing container (FIG. 10A). The wall of the container that is
20 configured to perform rotations is ball bearing (34) and is preferably circular.

The magnets may be arranged in various shapes in the raised portion of the magnetic separation station. In FIG. 11, the magnets (18) are arranged in two rows (35) separated from each other. The magnets in a row may be independently
25 operated from the magnets in the other row.

The present invention is in no way limited to the embodiments described in the examples and/or shown in the figures. On the contrary, methods according to the present invention may be realized in many different ways without departing from
30 the scope of the invention.

- 1- Purification or processing container
- 2- Inner wall
- 2'- Inner wall with minimum height
- 35 2''- Inner wall with maximum height
- 3- Outer wall
- 4- Processing space
- 4'- Inner processing space

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- 4"- Outer processing space
- 5- Knob
- 6- Central cavity
- 7- Indentation of the purification container
- 5 8- Distance between the outer and inner walls
- 9- Bottom wall
- 10'- maximum level of liquid sample
- 10"- minimum level of liquid sample
- 11- mixing station
- 10 12- base of the mixing station
- 13- raised portion of the mixing station
- 14- motor unit
- 15- indentation of the mixing station
- 16- magnetic separation station
- 15 17- base of the magnetic separation station
- 18- magnets lining the raised portion
- 19- screw holes
- 20- raised portion of the magnetic separation station
- 21- indentation of the magnetic separation station
- 20 22- system for nucleic acid production and purification
- 23- plurality of reaction chambers
- 24- pooling vessel
- 25- robotic arm
- 26- support rack
- 25 27- adaptor
- 28- needle
- 29- tube
- 30- waste or reactive vessel
- 31- lid
- 30 32- screws
- 33- magnets positioned at the base of the magnetic separation station
- 34- ball bearings
- 35- row of magnets

CLAIMS

1. A method for processing a liquid sample comprising one or more biochemical products, wherein said processing comprises:
 - 5 combining at least a fraction of said liquid sample with a plurality of magnetic particles to form a reactant mixture in a processing container, wherein the plurality of magnetic particles are able to bind to at least a portion of said biochemical products in the liquid sample;
 - 10 applying a magnetic source to the processing container, wherein biochemical products bound to the magnetic particles are separated from an unbound fraction of the liquid sample;
 - removing at least a portion of said unbound fraction and retaining the plurality of magnetic particles bound to the biochemical products; and
 - 15 optionally washing the retained magnetic particles with a wash buffer characterized in that the processing container comprises a wall structure, and a cavity, wherein said wall structure comprises at least an inner wall and an outer wall to form a processing space between said outer wall and said inner wall wherein said wall structure is configured to receive and retain a liquid sample into said inner space during processing of said liquid, wherein a
 - 20 distance between the inner wall and outer wall is less than 80 mm.
2. The method according to claim 1, wherein the distance between the inner wall and outer wall is between 3 and 75 mm.
3. The method according to any of claims 1 to 2, wherein an amount of magnetic particles is added or is present between the outer and inner wall of
- 25 said processing container.
4. The method according to any of the claims 1 to 3, further comprising mixing the magnetic particles and biochemical products in said processing container by subjecting said processing container to a mechanical motion.
5. The method according to claim 4, wherein the mechanical motion comprises
- 30 positioning said processing container on a mixing station, wherein said cavity engages with said mixing station.
6. The method according to any of the previous claims, wherein the applying the magnetic source step occurs at a magnetic separation station comprising one or more magnets, and further comprises said processing container
- 35 comprising said magnetic particles and biochemical product sample so that said magnetic particles are attracted to said magnet during a predefined magnetization time.

7. The method according to any of the previous claims, further comprising moving the processing container between the mixing station and the magnetic separation station by a robotic arm, configured to grasp said processing container.
- 5 8. The method according to any of the claims 2 to 7, wherein said biochemical products are produced in a plurality of reactions, preferably a plurality of in vitro transcription reactions, each reaction resulting in a biochemical product sample.
9. The method according to claim 8, wherein at least a portion of the plurality of biochemical product samples produced are pooled in a pooling vessel.
- 10 10. The method according to claim 9, wherein at least a portion of the pooled biochemical molecules from said pooling vessel is transferred to the processing container and subjected to a processing step such as a purification step.
- 15 11. The method according to any of the claims 9 or 10, wherein a further portion of said pooled samples is transferred to the processing container comprising biochemical products bound to magnetic particles and subjecting said portion to a binding step.
12. The method according to claim 11, wherein the magnetization time of the magnetic particles to the one or more magnets increases with the number of processing cycles.
- 20 13. The method according to any of the previous claims, wherein the biochemical products are nucleic acid molecules, preferably RNA or DNA.
14. A system for processing biochemical products by means of magnetic particles, wherein said system comprises:
one or more processing containers;
one or more mixing stations configured to receive one or more processing containers and comprising a motor unit configured to drive a mechanical motion of said processing container;
30 one or more magnetic separation stations comprising one or more magnets, wherein said processing container comprises a wall structure, defining a central cavity, wherein said wall structure comprises an outer wall and an inner wall defining a processing space between said outer wall and said inner wall and wherein said wall structure is configured to receive and retain a liquid sample into said inner space.
- 35 15. The system according to claim 14, wherein the distance between the outer wall and inner wall of said processing container is less than 80 mm, preferably between 3 and 75 mm.

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16. The system according to any of claims 14 or 15 wherein, the processing container comprises a bottom wall, wherein said bottom wall has an inclination causing a difference in liquid level of the liquid present in the inner space of the processing container.
- 5 17. The system according to any of the claims 14 to 16, wherein the one or more mixing stations and/or magnetic separation stations comprise a raised portion configured to receive the central cavity of said processing container.
18. The system according to claim 17, wherein the raised portion of the magnetic separation station comprises one or more magnets lining said raised portion.
- 10 19. The system according to any of the claims 14 to 18, wherein the magnetic separation station comprises a base comprising one or more magnets positioned at said base.
20. The system according to any of the claims 14 to 19, wherein the system further comprises one or more robotic arms configured to grasp said processing container and transfer said processing container between said one or more mixing stations and one or more magnetic separation stations.
- 15 21. The system according to any of the claims 14 to 20, wherein the system comprises a pooling vessel for receiving the biochemical product prior to the processing. The system according to claim 14 further comprises a computer processor, wherein said computer processor comprises instructions to control at least a handling means and to calculate and control a magnetization time of the magnetic particles bound to the biochemical product and the one or more magnets.
- 20 22. A processing container comprising a central cavity lined by a wall structure, wherein said wall structure comprises an outer wall and an inner wall defining an inner space between said inner and outer wall, wherein said wall structure is open at the top of said container, allowing a component and/or liquid to be added in the inner volume.
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