Currently established manufacturing processes for RNA molecules approved by regulatory authorities implement many separate manufacturing steps. Particularly, the respective manufacturing steps are performed by several different devices. Further, various separate quality controls are performed on DNA level and RNA level as described in detail in WO2016/180430A1.

A critical step in RNA production is the generation of a suitable DNA template, which at industrial scale is a major cost factor. Currently, DNA templates can only be used for a single RNA in vitro transcription reaction and need subsequently be destroyed by DNAse digestion and eventually removed by RNA purification in order to ensure efficacy and safety of the RNA-based therapeutics.

Manufacturing of RNA requires a large degree of manual handling in a GMP-regulated laboratory executed by well-trained technical staff. In consequence, current established manufacturing processes are time consuming, cost intensive, and require a lot of laboratory space and laboratory equipment.

Summary

As outlined above, there is the problem associated with common manufacturing devices and processes that RNA in vitro transcription currently requires a large degree of manual handling of well- trained technical staff. Thus, there is a need for providing an improved bioreactor for RNA in vitro transcription and an automated apparatus for RNA production to save time, space, equipment and personal.

An advantage of an improved bioreactor may be that it may allow for repetitive use of DNA templates in several RNA production processes which reduces the costs as less starting material (that is DNA template) has to be used and DNAse treatment can be omitted or substantially minimized. Moreover, an improved bioreactor may allow for the robust production of RNA with a higher purity profile (no residual DNAse, no residual DNA fragments in final RNA product). Advantages of an automated apparatus for RNA production are that the whole manufacturing process may be more robust and reliable (due to minimizing human error) and that the production of RNA may be accelerated.

Further, an acceleration of RNA manufacturing would be highly advantageous and of major importance for public health, especially in the context of pandemic scenarios. Further advantageous in that context would be the production of the RNA therapeutics in the region of the outbreak which would, however, require a portable RNA production apparatus.

The above problems are solved by the subject-matter of the independent claims, wherein further examples are incorporated in the dependent claims. It should be noted that the features of the disclosure described in the following apply equally to the bioreactor for RNA in vitro transcription, the method for RNA in vitro transcription, the module for transcribing DNA into RNA, the automated apparatus for RNA manufacturing and to the uses described herein.

In one aspect, the present disclosure refers to bioreactor (1) for RNA in vitro transcription comprising: (a) a reaction vessel (2) suitable to hold magnetic particles, DNA templates, a DNA immobilization buffer, DNA magnetic particles and an IVT master mix, wherein the DNA magnetic particles are DNA templates immobilized on the free-floating magnetic particles, and wherein the IVT master mix comprises ribonucleoside triphosphates and DNA dependent RNA polymerase, (b) a magnet unit (3) positioned at the reaction vessel, wherein the magnet unit is configured to capture or to introduce a movement of the magnetic particles and the DNA magnetic particles.

In a first example, the present disclosure is directed to a bioreactor for RNA in vitro transcription comprising:

- a reaction vessel, and
- a magnet unit positioned at the reaction vessel.

The reaction vessel is suitable to hold at least one of magnetic particles, DNA templates, a DNA immobilization buffer, DNA magnetic particles and an RNA in vitro transcription (IVT) master mix. Thereby, the DNA magnetic particles are DNA templates immobilized on the free-floating magnetic particles. The magnet unit is configured to capture or to introduce a movement of the magnetic particles and the DNA magnetic particles hold in the reaction vessel. With such movement, a mixing or stirring of the magnetic particles and/or the DNA magnetic particles can be induced. Accordingly, depending on the number of additional components hold in the reaction vessel, a mixing or stirring of magnetic particles and/or DNA magnetic particles as well as at least one of DNA templates, a DNA immobilization buffer, and an IVT master mix can be induced by the magnetic unit. For instance, with DNA templates and free-floating magnetic particles as components hold in the reaction vessel, a mixing or stirring of the magnetic particles induced by the magnet unit may lead to mixed DNA magnetic particles, wherein the DNA magnetic particles are the DNA templates immobilised on the magnetic particles. In case the DNA magnetic particles and the IVT master mix are mixed or stirred due to a movement of the DNA magnetic particles induced by the magnetic unit, the thereby established more homogeneous mixture of DNA magnetic particles and the IVT master mix supports the RNA in vitro transcription of template DNA into RNA.

The bioreactor according to the present disclosure may further be suitable for a use under regulated conditions (GMP) suitable for pharmaceutical applications (e.g. pharmaceutical nucleic acid production). The bioreactor may allow a continuous production or repeated batch production of a liquid nucleic acid composition, preferably a ribonucleic acid (RNA) composition. In the context of the disclosure, the term RNA is used to indicate any type of ribonucleic acid. Accordingly, the term "RNA" may refer to a molecule or to a molecule species selected from the group consisting of long-chain RNA, coding RNA, non-coding RNA, single stranded RNA (ssRNA), double stranded RNA (dsRNA), linear RNA (linRNA), circular RNA (circRNA), messenger RNA (mRNA), RNA 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), circular RNA (circRNA), and a Piwi-interacting RNA (piRNA).

In an example, the inner surface of the reaction vessel has an ellipsoid or an oval inner geometry.
It was found by the inventors, that an ellipsoid shape or oval inner geometry allows for a better mixing result. Additionally, such shapes allow for a better drip off or drain of fluids and may allow for better cleanability. The latter may prevent the formation of drops which otherwise could disadvantageously dry at the inner surface of the bioreactor. This may especially apply to e.g. proteinaceus residues of the fluid hold by the reaction vessel, which may e.g. harden or solidify at a temperature of 37°C or higher.

In an example, the inner surface of the reaction vessel has an egg-shape inner geometry. Such egg-shape may provide the same or improve the advantages as described above in context with the ellipsoid shape. An egg-shape may also provide for an optimal pressure distribution, optimal behaviour of the magnetic beads during mixing or steering, for holding the magnetic beads at the reaction vessel inner surface, distribution during cleaning process. An egg-shape may, for instance, be obtained from two half-spheroids with the same base radius, wherein one of the spheroids is a half-sphere with height equal to the base radius and the other spheroid has a height larger than the base radius. Alternatively, the inner surface of the reaction vessel may have a spheroidal-shape, in particular a shape of a sphere, or the inner surface may have a pill form. The inner surface of the reaction vessel may also have a form from a combination of an egg-shape and an ellipse or a combination

of an egg-shape and a cylindrical shape. By such combination, one part of the inner surface of the reaction vessel has e.g. an egg-shape, while the remaining part of the inner surface has e.g. a cylindrical shape.

In an example, the inner surface of the reaction vessel may have a spherical-shape inner geometry. Such spherical-shape may provide the same or improve the advantages as described above in context with the egg-shape reaction vessel.

In an example, the inner surface of the reaction vessel has a shape without edges (e.g. a cuboid with rounded edges). This shape likewise supports an optimal drain of drops and thereby prevents hardening of proteinaceous residues of the fluid hold in the reaction vessel. Such a shape (no edges) allows for an effective cleaning procedure.

In an example, the reaction vessel may have an inner surface without wide gaps or clefts. In that context, a gap or cleft larger than 2µm, preferably a gap or cleft larger than 1µm, more preferably a gap or cleft larger than 0.8µm is still considered to be a "wide" gap or cleft. Such a shape (no wide gaps) allows for an effective cleaning procedure as larger gaps may provide a niche for microbial contamination and biofilms or residues.

In an example, the movement of the magnetic particles and/or the DNA magnetic particles is configured such that a sedimentation of the particles hold in the reaction vessel is avoided. Additionally or alternatively, the movement of the magnetic particles and/or the DNA magnetic particles is configured to keep the particles comprised on the reaction vessel free-floating in such a way that a sedimentation at the reaction vessel's bottom can be prevented. Further, a mixing or swirling process is improved by keeping the particles in the vessel free-floating and/or that coagulation of beads is prevented or reduced. Advantageously, keeping magnetic particles and/or the DNA magnetic particles free floating and/or avoiding sedimentation of magnetic particles and/or the DNA magnetic particles improves biochemical reactions in the bioreactor, namely DNA immobilization and RNA in vitro transcription.

In an example, the magnet unit of the bioreactor is given by an array of electromagnets. The latter may be positioned on or in proximity to the outer surface of the reaction vessel. Individual electromagnets out of the array may be individually switched on or off. In such a way a mixing or swirling of magnetic particles and/or DNA magnetic particles hold in the reaction vessel may be improved and better controlled. Said array of electromagnets is preferably not movable and the bioreactor itself is not movable (no shaking etc.) and mixing or swirling is introduced by a cooperation of magnetic particles and/or DNA magnetic particles and the magnet unit.

The magnet unit may alternatively, in another example, be a permanent magnet or an electromagnet, which is movable in a longitudinal direction along a longitudinal axis of the reaction vessel. In addition or instead of such longitudinal movement, the permanent magnet or electromagnet may be movable in a transversal direction, towards and apart from the reaction vessel. Similarly to the case of an array of electromagnets, a longitudinally and/or transversally movable permanent or electromagnet may allow for a better control of mixing/swirling and a better mixing result.

The magnet unit may alternatively, in yet another example, be given by an electromagnet and preferably by at least one induction coil. In this case, the magnet unit is movable in a longitudinal direction along a longitudinal axis of the reaction vessel. In addition, the magnet unit is rotatable around a vertical axis of the reaction vessel.

Suitably, the magnet unit may be arranged in form of at least one Helmholtz coil.

A position of the magnet unit in proximity of the reaction vessel refers to a distance between magnet unit and reaction vessel, which still allows for a suitable magnetic field to be established inside the reaction vessel when the magnet unit is turned on. Thereby, the strength and the form of the magnetic field have to be such that a swirling/mixing of magnetic particles may be induced and/or magnetic particles may be captured on the inner surfaces of the reaction vessel.

In an example, the magnet unit is configured to rotate around a longitudinal axis of the reaction vessel, wherein a rotation direction of the magnet unit is switchable during mixing. The magnet unit may introduce a movement of the magnetic particles in a radial direction of the reaction vessel by inducing the magnetic particles in a radial direction relative to the longitudinal axis of the reaction vessel. The magnetic force can be static or dynamically generated by rotating the magnet unit around the reaction vessel to cause a rotation, accordingly mixing of the magnetic particles. Rotation direction of the magnet unit may be clockwise or anticlockwise relative to the longitudinal axis of the reaction vessel and/or alternately changed. Accordingly, the magnetic particles may stay free floating in a contactless manner, hence mixing of the components may be improved. As soon as the rotation of the magnet unit stops, the magnet particles (e.g. DNA magnetic particles) are captured at the inner surface of the reaction vessel and do not rotate any more. Accordingly, the magnet unit is configured to (i) rotate around a longitudinal axis of the reaction vessel to introduce a movement of the magnetic particles as explained above and configured to (ii) capture the magnetic particles when stopping rotation.

In an example, the magnet unit comprises a magnetic ring, wherein the magnetic ring is designed to surround the reaction vessel. To facilitate assembling and rotating of the magnet unit around the reaction vessel, the magnet unit may be formed in a ring shape. In other words, the reaction vessel may be positioned in a centre of a ring-shaped magnet unit such that the magnet unit encircles the reaction vessel.

In an example, the magnetic ring comprises at least a fist rod and a second rod extending from an inner circumference of the magnetic ring to a centre of the magnetic ring, so that the free ends of the first and second rods face each other. In an example, the free end of the first rod comprises a magnet with an N pole and the free end of the second rod comprises a magnet with an S pole.

The disc- or ring-shaped magnet unit may comprise a magnet arranged in a circumferential direction of the magnetic ring. The magnet may be arranged directly at and in contact with the magnetic ring or offset from the magnetic ring closer to the reaction vessel positioned in the centre of the magnet ring to reduce a gap between the magnet and the reaction vessel. To hold the magnet apart from the ring, a magnet holder connected to an inner surface and extending to the centre of the ring may be used. The magnet holder may be designed as a holding rod such that one end of the holding rod is attached to the inner circumference of the magnetic ring and the other end of the holding rod holds the magnet. The magnetic ring and the holding rods may be separately produced and attached to each other or manufactured as one piece, for example, by moulding.

To effectively induce a movement of the magnetic particles, the magnetic ring may comprise at least two rods spaced apart from each other along the circumference of the magnetic ring such that the free ends of the rods face each other. Further, to each free end of the rods a permanent magnet with an N pole and an S pole may be alternately attached. Accordingly, when rotating the magnetic ring, the magnetic particles may be rotatably induced around the reaction vessel, which causes an improved mixing of the components in the reaction vessel.

To effectively capture magnetic particles, rotation of the magnetic ring may be stopped after mixing the components in the reaction vessel.

In another example, the magnetic ring comprises a plurality of rods, wherein the plurality of the rods extend from an inner circumference of the magnetic ring to a centre of the magnetic ring and are arranged in a star shape evenly spaced apart from each other. Preferably, a magnet with an N pole and a magnet with an S pole are arranged alternately at each free end of the rods.

In a preferred example, the magnetic ring may comprise an even number of rods such that the plurality of rods, and accordingly the plurality of magnets attached to each free end of the rods are arranged in a paired manner to provide a heterogeneous or periodic magnet field. Further, the evenly along the circumference of the magnetic ring spaced rods allow a symmetric magnet field inducing the magnet particles inside the reaction vessel.

In an example, the magnetic ring and the rods are configured to form a laminated stack for shielding periphery components from a magnet field. The magnetic ring and the rods may be made of a plurality of laminated electrical sheets, which are magnetisable. The laminated electrical sheet may comprise electrical steel and may be used for an electrical insulation. The laminated stack may screen the magnetic field generated by the permanent magnets attached to the free ends of the rods and influence no other devices besides the reaction vessel. Shielding of the magnetic field is particularly advantageous and allows the integration of the bioreactor in an apparatus comprising other devices/components that may be influenced by magnetic fields.

In an example, the magnetic ring comprises a plurality of guide plates extending from an inner circumference of the magnetic ring to a centre of the magnetic ring. Preferably, each guide plate comprises an electric coil configured for generating a magnetic field. The magnetic ring may comprise at least one, preferably a plurality of electromagnets generating magnet fields by an electromagnetic coil. The guide plate may be arranged in a star shape along the circumference of the magnet ring and extend to the centre of the magnet ring where the reaction vessel may be positioned. The electromagnetic coils enable the magnetic field to be quickly changed by controlling the amount of electric current.

In an example, the magnetic ring is arranged in a housing having cooling means. The cooling means may be integrated in the housing of the magnetic ring along the circumference of the magnetic ring to carry away heat caused by high currents passing through the electromagnetic coils. The cooling means may be a cooling channel in which a cooling medium such as water is circulated. The cooling means may preferably be integrated in magnetic rings comprising an electromagnetic coil. The cooling means may not be integrated in magnetic rings comprising permanent magnets (and not comprising an electromagnetic coil).

In an example, the magnet unit further comprises a first driving means configured to rotate the magnetic ring around a longitudinal axis of the reaction vessel and a second driving means configured to move the magnetic ring in a vertical direction along the longitudinal axis of the reaction vessel. The magnetic ring may be held by a frame which moves in the longitudinal direction of the reaction vessel. Accordingly, the magnetic field may be provided and changed both in the longitudinal direction and the radial direction of the reaction vessel when the magnet ring rotates and moves vertically, which may lead to an even better homogeneous mixing of the components in the reaction vessel.

The driving means for rotating the magnetic ring and the driving means for moving the magnetic ring in the vertical direction may be provided separately. The first driving means for rotating the magnetic ring may be arranged directly to the magnetic ring and positioned above the reaction vessel, whereas the second driving means for vertically moving the magnetic ring may be connected to the magnetic ring via the frame fixedly holding the magnet ring and allowing the magnetic ring to move vertically.

In an example, the reaction vessel is paramagnetic such that magnetic particles and DNA magnetic particles may be withhold on the inner reaction vessel wall by a cooperation of the paramagnetic vessel and the magnet unit positioned at the reaction vessel. Thereby, the whole reaction vessel may be paramagnetic, or the inner surface of the reaction vessel may be paramagnetic, e.g. by comprising a paramagnetic material or a magnetically conductive material. The term "magnetisable" denotes throughout the disclosure that the reaction vessel or its inner surface may be temporarily magnetized such that magnetic particles may be attracted and withhold at the reaction vessel wall. The magnetization of the reaction vessel or its inner surface may however be reversed, such that magnetic particles and DNA magnetic particles withhold at the reaction vessel wall may be released. It is therefore important that the material of the bioreactor and/or the inner surface of the bioreactor are not permanently magnetized by switching on the magnet unit (that is, not ferromagnetic).

Accordingly, in a preferred example, the reaction vessel is paramagnetic. In other examples, the reaction vessel is configured to allow penetration of a magnetic field without being magnetisable.

In an example, the magnet unit is configured to be periodically active to mix the magnetic particles and/or the DNA magnetic particles. A periodic activation of the magnet unit may lead to an improved mixing of the components as compared to a continuous activation of the magnet unit. Such periodic activation of the magnet unit leading to an improved mixing of the components has to be adjusted in a way to keep the magnetic particles or the DNA magnetic particles free floating, and to allow a mixing in such a way that biochemical reactions occur in an optimized manner (all components involved in the biochemical reaction, e.g. in the RNA in vitro transcription are mixed and get in contact to each other that RNA synthesis occurs). It is likewise important to adjust the mixing induced by the periodically active magnet and the DNA magnetic particles/ magnet particles in a way that unwanted shear forces are minimized and that heat development is reduced (heat development may be induced by transformation of magnetic energy into heat, or induced by friction heat).

In an example, the magnet unit is configured to be activated to capture the DNA magnetic particles between two or more subsequent RNA in vitro transcriptions on the same DNA templates (provided in form of DNA magnetic particles). Such capture may be associated with a magnetization of the reaction vessel which leads to

withholding the DNA magnetic particles at the inner surface of the reaction vessel and/or may be associated with a magnetization of magnetisable but chemically inert beads or spheres within the reaction vessel Advantageously, such capture allows for a re-use of DNA magnetic particles in two or more RNA in vitro transcription reactions and thereby reduces time of production by decreasing template provision scale and costs of the RNA product (DNA template can be used several times).

In an example, the magnet unit is configured to be activated to remove the magnetic particles and DNA magnetic particles. Such removal of magnetic particles and DNA magnetic particles may be intended for a cleaning of the reaction vessel. The removal of DNA magnetic particles may be performed after the last RNA in vitro transcription reaction (e.g. by pausing the rotation of the magnetic ring). Such removal of DNA magnetic particle has the advantage that DNA can be removed without enzymatic digestion via e.g. DNAse which reduces DNA contaminations and enzyme contaminations in the final RNA product (no DNA digestion products, no DNAse enzyme), and reduces costs of the RNA product (no control for DNAse contamination in end-product needed, no DNAse enzyme needed).

In an example, no mechanical motion introducing means for the magnetic particles and DNA magnetic particles are comprised. According to this example, there are no additional mechanical stirrers or agitators which can induce a mixing or stirring of the components hold in the reaction vessel, so that the mixing is only induced by the magnet unit. This is particularly advantageous in the context of the disclosure as mechanical motion introducing means positioned inside the reaction vessel may cause the formation of unwanted precipitations (e.g. precipitations on the mechanical stirring means). Moreover, the absence of mechanical motion introducing means also improves the cleaning of the bioreactor (reduced surface, no edges inside the reaction vessel).

In an alternative example, a mechanical motion introducing means for the magnetic particles and DNA magnetic particles are comprised in form of a shaker (e.g., orbital shaker), wherein the shaker is preferably positioned outside the reaction vessel.

In an example, a mixing or stirring of the components hold in the reaction vessel may be introduced by a combination of (i) cooperation of the magnetic particles and a magnet unit, (i) mechanical motion introducing means, and/or (iii) directing a process gas or a process fluid into the reaction vessel.

In an example, the reaction vessel comprises at least one flow breaker arranged at least partially along an inner surface of the reaction vessel in a longitudinal direction of the reaction vessel. The flow breaker may disturb a uniform flow of the components in the reaction vessel and thereby improves mixing. Moreover, the flow breaker may prevent sedimentation of the magnet particles when the magnet ring stops rotating and/or changes rotation direction. Accordingly, the flow breaker may be designed continuously without any groove, in particular in a horizontal direction perpendicular to a longitudinal direction of the reaction vessel, in which the magnetic particles may be accumulated.

The flow breaker may protrude from the inner surface of the reaction vessel in a radial direction of the reaction vessel and extend along a longitudinal direction of the reaction vessel. The flow breaker may continuously extend from a top portion to a bottom portion of the reaction vessel or comprise a plurality of elements arranged

separately from each other along the longitudinal direction of the reaction vessel. Accordingly, the flow breaker may comprise a plurality of protrusions which are preferably spaced apart from each other.

In an example, the reaction vessel comprises two flow breakers spaced apart from each other along the circumference of the reaction vessel. The reaction vessel may comprise at least one, exactly two or more flow breakers. The flow breakers are preferably evenly distributed along the inner surface of the reaction vessel in the radial direction of the reaction vessel to improve mixing and to prevent sedimentation of the magnetic particles.

In an example, the flow breaker is rib-shaped and the rib-shaped flow breaker may preferably comprise a T- or L shaped cross section. The flow breaker protruding from the inner surface in direction to the centre of the reaction vessel may be formed in an arc shape along the curved inner surface of the reaction vessel and comprise a plurality of curvature radii along the ellipsoid inner geometry of the reaction vessel. A radial cross section of the flow breaker relative to the longitudinal axis of the reaction vessel may also vary. For instance, the radial cross section may be formed as a T-, L- or convex shape. A protrusion length of the radial cross section of the flow breaker may also vary along the inner surface from the top portion to the bottom portion of the reaction vessel. In an example, the flow breaker is corrugated. The rib-shaped flow breaker may be also wave-shaped along the inner surface of the reaction vessel, which may prevent a sedimentation of the magnetic particles. A wave-shaped surface of a corrugated flow breaker may be aligned perpendicular to the inner surface of the reaction vessel.

In an example, a temperature element is positioned between the inner surface and the outer surface of the reaction vessel for adjusting a temperature of the reaction vessel. In other words, the reaction vessel may comprises a thick wall made of a solid material allowing integration of the temperature element between the inner surface and the outer surface. Accordingly, a fast temperature adjustment regarding heating and cooling of the reaction vessel may be facilitated.

In an example, the temperature element comprises a heat exchange channel at least partially helically surrounding the reaction vessel in a radial direction of the reaction vessel. The heat exchange channel may be integrated between the inner surface and the outer surface and adapted to adjust the temperature in the reaction vessel. To provide an effective and uniform heating or cooling, the heat exchange channel may completely surround the reaction vessel and a heat exchange medium may flow inside the heat exchange channel.

In an example, the heat exchange channel comprises a first end and a second end, wherein the first end is arranged at a top portion of the reaction vessel and the second end is positioned at a bottom portion of the reaction vessel. The helically arranged heat exchange channel may have at least two ports for an inlet and/or an outlet of the heat exchange medium, wherein an efficient distribution of the heat exchange medium may be facilitated when one port is arranged at the top portion of the reaction vessel and the other port is arranged at the bottom portion of the reaction vessel, which may apply the gravitational force.

In an example, the heat exchange channel and/or the reaction vessel is manufactured by means of an additive manufacturing process. Accordingly, a complex geometry of the reaction vessel including the heat

exchange channel helically surrounding the reaction vessel between the inner surface and the outer surface of the reaction vessel may be easily realised.

In an example, the reaction vessel further comprises a temperature element, which comprises a heating wire at least partially helically surrounding the reaction vessel in a radial direction relative to a longitudinal axis of the reaction vessel. As an alternative to the heat exchange channel, the heating wire may be arranged on the reaction vessel to adjust the temperature of the components in the reaction vessel. The heating wire may also helically surround the reaction vessel to provide uniform heating.

In an example, the heating wire is at least partially integrated in an outer surface of the reaction vessel or at least partially coated on the outer surface of the reaction vessel. To minimize heat loss and to provide an efficient heating by the heating wire, the heating wire may be fixed to the outer surface of the reaction vessel. Alternatively or in addition to that, the outer surface of the reaction vessel may be coated with a heat isolation material and the heating wire may be at least partially retracted in the heat isolation material.

In an example, the reaction vessel is dimensioned such that it can uptake at least 20ml of fluid, or at least 50 ml of fluid, or at least 100ml of fluid, or at least 500ml of fluid. Preferably, it can uptake 20 ml to 100 ml or 20 ml to 50 ml of fluid. It may also be configured to uptake 50 ml to 100 ml of fluid. Of note, when used in a method as specified in the second example, said reaction vessel is filled to only about 60% to about 80% to allow sufficient shaking of the liquid. In a specific example, the reaction vessel is dimensioned such that it can uptake about 100 ml of fluid, wherein only 60 ml to 80 ml of fluid is filled into the reaction vessel, corresponding to a reaction vessel filled to only about 60% to 80%. In another specific example, the reaction vessel can uptake about 20 ml to 50 ml of fluid, wherein the reaction vessel shall be filled in this case to about 60%.

The IVT master mix may, according to an example, comprise ribonucleoside triphosphates and DNA dependent RNA polymerase. The DNA immobilisation buffer may, according to an example, comprise DNA and salt containing buffers. The DNA templates may be given by linear double stranded DNA templates, which are preferably PCR amplified DNA templates. In an example, the magnetic particles may be given by magnetic beads, preferably streptavidin magnetic beads or chemically functionalized magnetic beads, most preferably paramagnetic streptavidin or chemically functionalized magnetic beads.

In an example, the inner surface of the reaction vessel has a surface roughness value (Ra value) of Ra<=0.8, preferably Ra<=0.6. The inner surface may be, e.g., electro-polished or otherwise, e.g. chemically or mechanically, treated such that the aforementioned Ra values are achieved. Such Ra values are particularly advantageous as such a material may improve the cleanability of the reactor because it may prevent or reduce deposition and hardening of e.g. proteinaceous residues or biofilms at the inner surface of the reaction vessel.

In an example, the bioreactor comprises an inlet port, which allows for introducing a filling medium into the reaction vessel. Thereby, the inlet port is arranged below a maximal fluid amplitude or fluid level. In context of the present disclosure, a maximal fluid amplitude is understood to be the amplitude a fluid contained in the reaction vessel and brought into a shaking or rotational movement maximally reaches on the inner surface of the reaction vessel. In case of a rotational movement, centrifugal forces acting on the fluid molecules lead to a pushing of fluid upwards the inner surface of the reaction vessel. The boundary between moistened and dry

area on the inner surface defines a line, which gives the maximal fluid amplitude. In other words, a maximal fluid amplitude can be associated with a line or area which is moistened in course of a shaking or rotational movement of fluid contained in the reaction vessel. A filling medium to be introduced through the inlet port of the reaction vessel may e.g. be given by magnetic particles, DNA templates, an immobilization buffer and/or an IVT master mix. Further filling media may be cleaning, wash and process fluids or the like. Positioning the inlet port below a maximal fluid amplitude prevents deposition and hardening of substances (e.g. proteins, DNA, or particles, or salts etc.) at the inner surface of the reaction vessel, which may for instance be the case for protein at temperatures around 37°C.

In an example, the reaction vessel comprises a medium port at a bottom of the reaction vessel for supplying and/or removing medium into/out of the reaction vessel and the port is connectable to a valve means. In other words, the bioreactor comprises a combined inlet and outlet port (inlet/outlet port), preferably positioned at the lowermost point of the reaction vessel. The valve means may allow for introducing a filling medium into the reaction vessel and for draining the medium out of the reaction vessel. Advantageously, the valve means may be configured to keep e.g. the magnetic particles and DNA magnetic particles inside the reaction vessel when the valve means is closed, or to allow passage of e.g. fluids comprising RNA product when the valve means is open.

In an example, the valve means comprises a magnetic trap. The latter is positioned at the medium port and configured to catch magnetic particles and DNA magnetic particles. In such a way magnetic particles and DNA magnetic particles may be caught when cleaning the reaction vessel. Additionally or alternatively, magnetic particles and DNA magnetic particles which unwantedly left the reaction vessel may be caught and thereby separated from e.g. produced RNA. The magnetic trap may be positioned outside the reaction vessel and may at least partially surround a medium pipe. The latter pipe may be connected to and downstream abuts the medium port of the reaction vessel. The medium port may be positioned at the lowermost point of the reaction vessel. In that way, fluids may easily outflow the reaction vessel driven by the gravitational force.

In an example, the magnetic trap comprises magnetisable or magnetic spheres or magnetisable or magnetic rings and/or semi-permeable filters, which allow retaining magnetic particles and/or DNA magnetic particles. The magnetic trap may comprise an electromagnet or a permanent magnet. The magnetic trap may be controllable to prevent an escape of magnetic particles and or DNA magnetic particles from the reaction vessel. Such control can be advantageously used when separating produced RNA from magnetic particles and DNA magnetic particles.

In an example, the bioreactor comprises a multi position valve. The latter is positioned downstream the magnetic trap and configured for directing a cleaning gas or a cleaning fluid through the port. This configuration serves to remove magnetic particles and DNA magnetic particles or other sedimentation collected at the port from the latter.

In an example, the aforementioned multi position valve is configured to direct a process gas or a process fluid into the reaction vessel. The process gas or process fluid directed into the reaction vessel may lead to a mixing, stirring or swirling of the magnetic particles or DNA magnetic particles.

In an example, the bioreactor comprises a valve means positioned at the outlet port, the inlet port, and/or the inlet/outlet port and is configured to keep e.g. the magnetic particles and DNA magnetic particles inside the reaction vessel when the valve means is closed, or to allow passage of e.g. fluids comprising RNA product when the valve means is open. Advantageously, the valve means may be configured to allow closing and opening of the outlet port and/or inlet port or the combined inlet/outlet port. Suitably, such a valve means may be a ball valve, a butterfly valve, a control valve, a diaphragm valve, a gate valve, a needle valve or a pinch valve or combinations thereof.

In an example, the bioreactor comprises at least a first leg and a second leg vertically (along a longitudinal direction of the reaction vessel) supporting the bioreactor. The first leg comprises a first conduit and the second leg comprises a second conduit. The first conduit is configured to be in a fluid communication with the valve means and the second conduit is configured to be in a fluid communication with one end of the heat exchange channel of the temperature element. The first leg and the second leg may be positioned at the bottom portion of the reaction vessel and configured to vertically stabilise the reaction vessel. Moreover, the first and the second leg may comprise a conduit within the respective legs. The first end of the first conduit located in the first leg may be connected to the valve means to supply and/or drain the reaction components and the second end of the first conduit may be connected to a periphery device supplying and /or draining the reaction medium. Further, the first end of the second conduit located in the second leg may be connected to the second end of the heat exchange channel helically wound around the reaction vessel and the second end of the second conduit may be connected to a periphery device supplying and /or draining the heat exchange medium. Accordingly, the reaction vessel may be compactly designed.

In an example, the bioreactor comprises an exit port. The exit port is connected to at least one of an exhaust duct and a waste channel. For instance, the exit port may be connected to at least both the exhaust duct and the waste channel by a multi position valve. The exit port may allow for receiving and venting exhaust gas or exhaust gases emerging within the reaction vessel. In case of waste fluid or a cleaning fluid, the exit port may serve for draining the fluid out of the reaction vessel. The exit port, the exhaust duct and/or the waste channel may hold at least one means for measuring and/or adjusting pressure.

In an example, the bioreactor further comprises a Hall sensor. The latter is configured downstream the magnetic trap and serves to detect magnetic fields. The Hall sensor may watch or control that products, e.g. fluids, entering a capillary downstream the magnetic trap are free of magnetic particles and/or DNA magnetic particles. In this way, the Hall sensor helps to control the correct operation of the magnetic trap. As a consequence of a measurement of magnetic fields emerging from e.g. magnetic particles or DNA magnetic particles by the Hall sensor, a fault signal may be given.

In context of the present disclosure, "downstream" and "upstream" refer to a direction of motion of fluids or gases within the processes covered by the present disclosure. For instance, when a Hall sensor is configured downstream a magnetic trap, this implies that the magnetic trap and the Hall sensor are arranged at, e.g. surround, a capillary within which fluids or gases are conducted, and that a fluid or gas conducted within the capillary will first pass by the magnetic trap and afterwards pass by the Hall sensor.

In an example, the reaction vessel comprises Titan. Titan comprises a lower remanence, in other words residual magnetism, which indicates a magnetization left behind in a ferromagnetic material after an external magnetic field is removed. Accordingly, the reaction vessel made of titan may provide an immediate interaction between the magnet force generated by the magnet ring and the magnet particles contained inside the reaction vessel.

Suitably, the reaction vessel has a material that is resistant to e.g. cleaning procedures (chemically resistant), extreme temperatures (e.g. 75° and 85°C. for cleaning procedure), extreme pH values (cleaning of the reactor with bases and acids, e.g. with NaOH), mechanical forces (e.g. frictions caused by magnetic particles), and/or corrosion. Moreover, materials of the reaction vessel should be temperature conductive at working temperatures around 20°C (e.g. W/(mK) values of at least 10, preferably at least 15).

Suitably, and in the context of the disclosure of particular importance, the inner surface of the reaction vessel has a surface material that does not release unwanted compounds that may contaminate the end product. Suitable materials of the reaction vessel and/or the inner surface of the reaction vessel are austenitic stainless steel (e.g., 1.4404 (AISI 316L), 1.4435 (AISI 316L)), iron-less Hastelloy® alloys or titan (Ti1), which being paramagnetic, chemically resistant, pH resistant, temperature resistant, and temperature conductive.

Further suitable materials of the reaction vessel and/or the inner surface of the reaction vessel are 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 non-magnetizable, chemically resistant, pH resistant, and temperature resistant. An advantage of glass (e.g. borosilicate glass) may be that the reaction vessel may be inspected visually.

In an example, the bioreactor further comprises a (semi permeable) filter element at the medium port or the medium pipe. The filter may help to withhold magnetic particles and DNA magnetic particles within the reaction vessel. The filter element may have a pore size smaller than 1 µm for an effective filtering. The semi-permeable filter may comprise a filter membrane with a molecular weight cutoff (MWCO) suitable for withholding magnetic particles and/or DNA magnetic particles. For preventing a clogging of the port by a clogged filter, the filter may preferably be a single use filter.

In an example, the temperature element is configured to adjust the temperature within the reaction vessel to a DNA immobilization or RNA transcription temperature of 20°C to 37°C. In addition, the temperature element may also be configured to adjust the temperature within the reaction vessel to a cleaning temperature of 75°C to 85°C. The suitable temperature (e.g. 20°C to 37°C or 75°C to 85°C) may be controlled by at least one means for measuring and/or adjusting temperature (e.g. a temperature sensor), said at least one means may suitable be positioned at the inner surface of the reaction vessel and/or in proximity to the reaction vessel and/or in proximity to the temperature element. A temperature element and a means for measuring and/or adjusting temperature is particularly important as e.g. magnetic energy or friction may produce unwanted heat that may impede biochemical reactions (e.g. unwanted temperature increase in reaction).

In an example, the bioreactor comprises an inlet flow cell and/or an outlet flow cell and/or an exit flow cell. The inlet flow cell may be arranged upstream the inlet port and the outlet flow cell may be arranged downstream

the outlet port and the exit flow cell may be arranged downstream the exit port. The inlet flow cell and/or the outlet flow cell and/or the exit flow cell may be calibratable and may be arranged for monitoring a flow rate of fluids or gases flowing into or out of the reaction vessel. The latter may contribute to a partial control of the processes of e.g. RNA transcription or cleaning of the bioreactor in context of the present disclosure.

In an example, the reaction vessel is configured to additionally hold at least one of the following elements given by a buffer suitable for RNA in vitro transcription, ribonucleoside triphosphates, a cap analogue, modified ribonucleoside triphosphates, a ribonuclease inhibitor, a pyrophosphatase, MgCl2, an antioxidant, a polyamine and a solution for cleaning and/or sanitizing.

In an example, the reaction vessel may be further configured to hold at least one means for measuring and/or adjusting pH or concentration of contained components, as well as a magnesium concentration, phosphate concentration, temperature, pressure, flow velocity, RNA concentration and/or ribonucleotide triphosphate concentration. The means may be given by a respective sensors or a respective probe. Such mean or means may contribute to a monitoring of the processes covered by the present disclosure. The measuring means may be a measuring device or sensor and the adjusting means maybe be a dosage device. For instance, the means may be a sensor for measuring the pH of components contained in the reaction vessel, or a sensor for measuring the magnesium or salt concentration. Further, exemplarily, the means may be a device for measuring the temperature, pressure or flow velocity. In the latter case, the means may be, for instance, a flow-cell inside or at an outflow of the reaction vessel.

In an example, the bioreactor is designed to operate in batch, a repeated batch, continuous mode or in a semi-continuous or continuous mode. Repeated batch RNA in vitro transcription (IVT) is preferred as it allows several reactions on the same DNA template with the advantages as already outlined herein.

In an example wherein the bioreactor comprises mechanical motion introducing means, the bioreactor may comprise rotation means for rotating the reaction vessel. Such rotation may help to prevent a sedimentation of magnetic particles and DNA magnetic particles at the outlet port.

In another aspect, the present disclosure refers to a method for RNA in vitro transcription, wherein the method comprises the following steps: - providing magnetic particles, DNA templates, a DNA immobilisation buffer in a reaction vessel of a bioreactor (1) as disclosed herein (S1), - mixing the magnetic particles, the DNA templates and the DNA immobilisation buffer by means of a cooperation of the magnetic particles and a magnet unit of the bioreactor to obtain DNA magnetic particles, which are the DNA templates immobilized on the free-floating magnetic particles (S2), - providing DNA magnetic particles and IVT master mix in the reaction vessel of the bioreactor (1), - mixing free-floating DNA magnetic particles with the IVT master mix by means of a cooperation of the DNA magnetic particles and the magnet unit to obtain RNA (S3).

In a second example, the present disclosure is directed to a method for RNA in vitro transcription. The method comprises the following steps:

- providing DNA magnetic particles and IVT master mix in a reaction vessel of a bioreactor, wherein the bioreactor is designed according to at least one of the above described examples of the first example (S3a),
- mixing free-floating DNA magnetic particles with the IVT master mix by means of a cooperation of the DNA magnetic particles and the magnet unit of the bioreactor and/or by means of a shaker. To this end, the magnet unit may be configured to induce a movement of the DNA magnetic particles and the components of the IVT master by appropriate electromagnetic fields. As a result of the mixing, in vitro transcribed RNA is obtained (S3b).

The method of the second example may additionally comprise the following steps:

- providing magnetic particles, DNA templates, a DNA immobilization buffer in a reaction vessel of a bioreactor wherein the bioreactor is designed according to at least one of the

above described examples of the first example (S1),

- mixing the magnetic particles, the DNA templates and the DNA immobilization buffer. Mixing is performed by means of a cooperation of the magnetic particles and a magnet unit and/or by means of a shaker (S2). To this end, the magnet unit may be configured to induce a movement of the magnetic particles and DNA magnetic particles by appropriate electromagnetic fields. As a result of the mixing, DNA magnetic particles, which are the DNA templates immobilized on the free-floating magnetic particles, are obtained. Said DNA templates immobilized on the free-floating magnetic particles may be mixed with IVT master mix to obtain RNA (as described above; S3). Accordingly, steps S1 and S2 are performed prior to the step S3.

The method of the second example may additionally comprise the following steps:

- capturing DNA magnetic particles by means of the magnet unit and collecting/harvesting obtained in vitro transcribed RNA e.g. through the outlet port (S4a),
- providing fresh IVT master mix in a reaction vessel of a bioreactor of the first example (S4b),
- releasing captured DNA magnetic particles to provide free-floating DNA magnetic particles (S4c),
- mixing the free-floating DNA magnetic particles with the IVT master mix by means of a cooperation of the DNA magnetic particles and the magnet unit and/or by means of a shaker to obtain RNA (S4d).

Preferably, steps S4a-S4d are performed after step S3. Said method steps (S4) are particularly suitable in examples where more than one RNA in vitro transcription reaction is performed. Preferably, said method steps are performed at least 2 times, e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10 or up to 30 times.

In an example, the method according to the present disclosure may further comprise the step of adjusting the pH and/or salt concentration.

In an example, the method according to the present disclosure further comprises the step of tempering the reaction vessel to a temperature between 20°C and 37°C. Such temperature may be suitable for RNA in vitro transcription. The tempering may be performed before filling the reaction vessel.

The reaction vessel may be tempered to a temperature between 20° and 25°C, preferably 22°C, in an additional method step for immobilisation of DNA templates on magnetic particles. Further, in an additional method step, the reaction vessel may be tempered to a temperature between 75° and 85°C for/during a cleaning process of the reaction vessel.

In an example, the method further comprises the step of cleaning the reaction vessel by a cleaning gas and/or cleaning fluid. Before or during cleaning, the reaction vessel may e.g. heated to an aforementioned temperature between 75° and 85°C. After obtaining the in vitro transcribed RNA, DNA magnetic particles may be removed by means of a cooperation of the DNA magnetic particles and the magnet unit. This method step allows removal of the DNA template without e.g. performing DNAse digestion.

To allow mixing or capturing/releasing of magnetic particles or DNA magnetic particles it is important that the magnetic particles are paramagnetic to avoid irreversible attachment to the wall of the reactor vessel during e.g. the mixing introduced by the magnet unit. Examples of suitable magnetic particles are Dynabead® magnetic beads (ThermoFisher Scientific).

The method may further comprise different quality control steps that may allow for assessment of e.g. RNA identity, RNA integrity, RNA purity etc. Said quality controls may be implemented in-line or at-line.

The method for RNA in vitro transcription as outlined herein may be performed on one DNA template to generate a RNA composition comprising one RNA species. In other examples, the method for RNA in vitro transcription may be performed on at least two different DNA templates to generate a composition comprising at least two RNA species. E.g., methods described in WO2017/1090134A1 may be adapted accordingly and performed in a reaction vessel of a bioreactor of the first example.

The method may further comprise a step of enzymatic RNA capping that may be performed in a reaction vessel of a bioreactor of the first example (e.g. using immobilized capping enzymes on magnetic particles; immobilized capping enzymes may be obtained using methods disclosed in WO2016/193226). The magnet unit may be configured to induce a movement of the capping enzymes on magnetic particles and the RNA by appropriate electromagnetic fields. As a result of the mixing, capped RNA is obtained.

The method may further comprise a step of enzymatic RNA Polyadenylation that may be performed in a reaction vessel of a bioreactor of the first example (e.g. using immobilized PolyA polymerases on magnetic beads immobilized PolyA polymerases may be obtained using methods disclosed in WO2016/174271). The magnet unit may be configured to induce a movement of the PolyA polymerases on magnetic particles and the RNA by appropriate electromagnetic fields. As a result of the mixing, polyadenylated RNA is obtained.

In yet another aspect, the present disclosure refers to use of a bioreactor (1) as disclosed herein in a method as disclosed herein.

In a third example, the present disclosure is directed to a use of a bioreactor as described above in a method as described above.

Further, the bioreactor of the first example may be used for RNA in vitro transcription reactions where the DNA is free or immobilized on non-magnetic particles (e.g. agarose beads, sepharose beads, non-magnetic polystyrol beads, and other appropriate synthetic resins) and the mixing is introduced by means of a cooperation of magnetic particles that do not carry DNA template and the magnet unit of the bioreactor of the first example. In such an example, the RNA in vitro transcription reaction can only performed once. Further, the bioreactor may be used in any enzymatic method involving nucleic acids (e.g., Polymerase Chain Reaction (PCR), isothermal DNA amplification, Reverse transcription of RNA into cDNA) wherein mixing is introduced by means of a cooperation of magnetic particles and the magnetic unit of the bioreactor of the first example.

In a fourth example, the present disclosure is directed to a module for transcribing template DNA into RNA. The module comprises a bioreactor according to at least one of the above examples of the first example, and further at least one of a unit for preparing an IVT master mix, a unit for preparing an immobilization buffer, a device for purifying an obtained RNA product, a device for RNA conditioning and/or a device for RNA sterile filtration.

In preferred examples, the device for purifying an obtained RNA product comprises an HPLC unit, preferably a unit for performing RP-HPLC. Particularly preferred in that context is RP-HPLC using a method disclosed in WO2008/077592 preferably using a porous, non-alkylated poly(stryrene-divinylbenzene) reverse phase, wherein the reverse phase is formed by beads or occurs as a polymerized block (e.g. monolithic). Alternatively, or in addition, the device for purifying an obtained RNA product may comprise an oligo dT purification unit for affinity purification of obtained polyadenylated RNA via oligo dT functionalized matrices or beads or columns (e.g. as described in WO2014152031A1).

In preferred examples, the device for RNA conditioning comprises a tangential-flow filtration unit. Particularly preferred in that context is tangential flow filtration as described in WO2016/193206, wherein TFF is used for diafiltration and/or concentration and/or purification of RNA.

In an example, the module further comprises a media supply unit. The latter unit is configured to supply components of the IVT master mix to the unit for preparing the IVT master mix.

In an example, the DNA template is an end-modified or end-functionalised PCR-generated DNA template. Preferably, the DNA template is a biotinylated PCR-generated DNA template, a non-modified or end-modified linearized plasmid DNA or a non-modified or end-modified linearized doggy bone DNA.

In yet another aspect, the present disclosure refers to an automated apparatus for RNA manufacturing, comprising a bioreactor (1) as disclosed herein, the apparatus further comprising at least one of: - a module for DNA synthesis (T), and - a module for RNA formulation (F).

In a fifth example, the present disclosure is directed to an automated apparatus for RNA manufacturing, comprising an aforementioned bioreactor of the first example or a module of the forth example, wherein the apparatus further comprises at least one of a module for DNA synthesis and a module for RNA formulation.

In an example, the module for DNA synthesis is configured to generate sufficient amount of DNA suitable for use in a bioreactor of example 1 or the module for transcribing DNA template into RNA of example 2. In a preferred example, the module for DNA synthesis may comprise a thermocycler element for PCR-based DNA amplification and an element for purifying obtained PCR products. Suitably, said module for DNA synthesis may generate biotinylated DNA templates, preferably PCR-based biotinylated DNA templates.

In an example, the module for RNA formulation is configured to generate lipid nanoparticle (LNP) encapsulated RNA. Accordingly, the module for RNA formulation comprises an LNP formulation module, wherein said LNP formulation module may comprise e.g. a pump element (e.g. a syringe pump), a tangential flow element, and a filtration element (e.g. comprising a sterile filter).

In an example, the module for RNA formulation is configured to generate an RNA complexed with a polycationic peptide or protein (e.g. protamine or a polymeric carrier, e.g. a polyethylene glycol/peptide polymer e.g. according to WO2012/013326). Accordingly, the module for RNA formulation comprises at least one of a Protamine formulation/complexation module and/or a polyethylene glycol/peptide polymer formulation/complexation module. In that context, the module for RNA formulation may suitably use methods and means according to WO2016165825A1 and/or WO2018041921A1.

In an example, the automated apparatus is arranged in a closed container and preferably in a single container, wherein the container comprises a unit for laminar airflow generation. Such configuration within a single container in particular helps to save space, in addition to equipment and personnel. Moreover, said automated apparatus may be configured to be portable, e.g. dimensioned in a way to allow transportation to regions of an outbreak of a pandemic.

In an example, the automated apparatus further comprises at least one of a DNA immobilization module e.g. for immobilizing plasmid DNA, e.g. as described in PCT/EP2017/084264 or PCT/EP2018/086684, a DNA linearization module e.g. for linearization of plasmid DNA or doggy bone DNA, a RNA capping module e.g. for adding a cap0 or cap1 structure to in vitro transcribed RNA, a RNA polyadenylation module e.g. for adding a polyA tail to in vitro transcribed RNA, an RNA mixing module e.g. for mixing at least two different RNA species, an RNA spray drying module e.g. for generating spray-dried or freeze-spray dried RNA e.g. according to WO2016/184575 or WO2016184576, an RNA lyophilization module for generating lyophilized RNA e.g. according to WO2016/165831 or WO2011/069586, and/or a module for end-product storage.

In an example, the automated apparatus further comprises at least one of an NGS (next generation sequencing) module e.g. for sequence analysis, a mass-spectrometry (MS) module, a quality control module (e.g. comprising an HPLC unit for analytical HPLC), a qPCR or ddPCR module, a capillary electrophoresis module, a media supply rack or a media supply module, a documentation module and/or a module for computer assisted control for all processing steps and interfaces for higher order controls and documentation systems.

In summary, devices and methods for the economical, controllable, reproducible, continuous (repeated batch), and GMP-compatible RNA production are presented. Described methods and means allow for repetitive use of DNA templates in several RNA (mass) production processes. The devices as described above therefore allow, for instance, for an accelerated production of RNA manufacturing. Further, an automated and, due to an appropriate size, portable RNA production apparatus as described above is advantageous in context of production of RNA therapeutics in a region of an outbreak of a pandemic.

It shall be understood that the bioreactor for RNA in vitro transcription, the method for RNA in vitro transcription, the use of a bioreactor according to the method, the module for transcribing DNA into RNA, and the automated apparatus for RNA manufacturing according to the independent claims have similar and/or identical preferred examples, in particular, as defined in the dependent claims. It shall be understood further that a preferred example of the disclosure can also be any combination of the dependent claims with the respective independent claim.

These and other aspects of the present disclosure will become apparent from and be elucidated with reference to the embodiments described hereinafter.

Brief description of the drawings

The Figures shown in the following are merely illustrative and shall describe the present invention in a further way. These figures shall not be construed to limit the present invention thereto.

CLAIMS

1. A bioreactor (1) for RNA in vitro transcription comprising:
   (a) a reaction vessel (2) suitable to hold magnetic particles, DNA templates, a DNA immobilization buffer, DNA magnetic particles and an IVT master mix,
   wherein the DNA magnetic particles are DNA templates immobilized on the free-floating magnetic particles, and
   wherein the IVT master mix comprises ribonucleoside triphosphates and DNA dependent RNA polymerase,
   (b) a magnet unit (3) positioned at the reaction vessel,
   wherein the magnet unit is configured to capture or to introduce a movement of the magnetic particles and the DNA magnetic particles.

2. Bioreactor (1) according to claim 1, wherein an inner surface of the reaction vessel (2) has an ellipsoid, an oval inner geometry or an egg-shape inner geometry, wherein the inner surface of the reaction vessel (2) has a shape without edges.

3. Bioreactor (1) according to claim 1 or 2, wherein the magnet unit (3) is a permanent magnet or an electromagnet movable in a longitudinal direction (362) along a longitudinal axis of the reaction vessel (2) and/or a transversal direction (363) towards and apart from the reaction vessel (2).

4. Bioreactor (1) according to one of the preceding claims, wherein the magnet unit (3) is configured to rotate around the longitudinal axis of the reaction vessel (2), and wherein a rotation direction of the magnet unit (3) is switchable during mixing.

5. Bioreactor (1) according to one of the preceding claims, wherein the magnet unit (3) comprises a magnetic ring (31), and wherein the magnetic ring (31) is designed to surround the reaction vessel (2).

6. Bioreactor (1) according to the preceding claim, wherein the magnetic ring (31) comprises at least a first rod (320) and a second rod (322) extending from an inner circumference (34) of the magnetic ring (31) to a centre (33) of the magnetic ring (31), so that free ends (321, 323) of the first and second rod (320, 322) face each other, and wherein the free end (321) of first rod (320) comprises a magnet with an N pole and the free end (323) of the second rod (322) comprises a magnet with an S pole.

7. Bioreactor (1) according to one of the preceding claims, wherein the reaction vessel (2) is paramagnetic or is configured to allow penetration of a magnetic field for withholding magnetic particles and DNA magnetic particles on the reaction vessel wall.

8. Bioreactor (1) according to one of the preceding claims, wherein a mechanical motion for the reaction vessel is introduced by an orbital shaker.

9. Bioreactor (1) according to one of the preceding claims, wherein the reaction vessel (2) comprises at least one flow breaker (4) arranged at least partially along an inner surface (21) of the reaction vessel (2) in a longitudinal direction of the reaction vessel (2).

10. Bioreactor (1) according to one of the preceding claims, wherein a temperature element (5) is positioned between the inner surface (21) and the outer surface (23) of the reaction vessel (2) for adjusting a temperature of the reaction vessel (2), wherein the temperature element (5) comprises a heat exchange channel (51) at least partially helically surrounding the reaction vessel (2) in a radial direction of the reaction vessel (2).

11. Bioreactor (1) according to one of the preceding claims 1 to 9, wherein the reaction vessel (2) further comprises a temperature element (5), which comprises a heating wire (54) at least partially helically surrounding the reaction vessel (2) in a radial direction of the reaction vessel (2), wherein the heating wire (54) is at least partially integrated in an outer surface of the reaction vessel (2) or at least partially coated on the outer surface of the reaction vessel (2).

12. Bioreactor (1) according to one of the preceding claims, wherein the reaction vessel (2) is configured for an uptake of at least 20 ml of fluid, preferably 20 ml to 100 ml or 20 ml to 50 ml of fluid.

13. Bioreactor (1) according to one of the preceding claims, further comprising valve means (60) with a multi position valve (62) positioned downstream a magnetic trap (61) and configured to direct a cleaning gas or cleaning fluid through the port (24) to remove magnetic particles and DNA magnetic particles from the port (24) and/or wherein the multi position valve (62) is configured to direct a process gas or process fluid into the reaction vessel (2) to mix the DNA magnetic particles.

14. A method for RNA in vitro transcription, wherein the method comprises the following steps:
- providing magnetic particles, DNA templates, a DNA immobilisation buffer in a reaction vessel of a bioreactor (1) according to any one of claims 1 to 12 (S1),
- mixing the magnetic particles, the DNA templates and the DNA immobilisation buffer by means of a cooperation of the magnetic particles and a magnet unit of the bioreactor to obtain DNA magnetic particles, which are the DNA templates immobilized on the free-floating magnetic particles (S2),
- providing DNA magnetic particles and IVT master mix in the reaction vessel of the bioreactor (1),
- mixing free-floating DNA magnetic particles with the IVT master mix by means of a cooperation of the DNA magnetic particles and the magnet unit to obtain RNA (S3).

15. Use of a bioreactor (1) according to any one of claims 1 to 13 in a method according to claim 14.

16. An automated apparatus for RNA manufacturing, comprising a bioreactor (1) according to any one of claims 1 to 13, the apparatus further comprising at least one of:
- a module for DNA synthesis (T), and
- a module for RNA formulation (F).