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(54) **MIXING UNIT, A MIXING SYSTEM COMPRISING TWO OR MORE MIXING UNITS , A METHOD FOR MIXING TWO OR MORE FLUIDS AND A LYSIS SYSTEM**

(57) The invention relates to a mixing unit (1) for mixing two or more fluids, in particular fluids containing shear sensitive biological material, the mixing unit comprising: a first inlet (4) for receiving an inlet stream, the inlet stream comprising a first fluid and a second fluid; a channel structure in fluid communication with the inlet, the channel structure comprising: a first branching (10) for splitting the inlet stream into a first (12-1) and a second (12-2) primary branch streams; a first (14-1) and a second (14-2) primary channel, each configured for piping of one of respective primary branch streams, a first collecting reservoir (18) for recombining the primary branch streams; wherein the first and second primary channels fluidly connect the first branching with the first collecting reservoir.

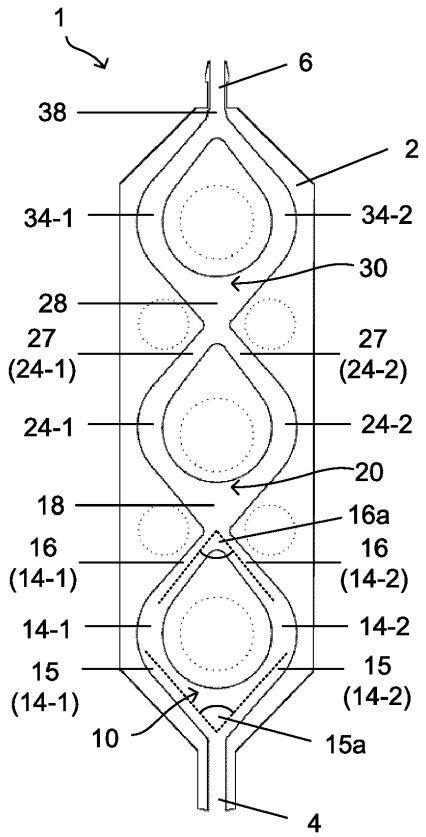

Fig. 2

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Description

[0001] The present disclosure relates to a mixing unit, a mixing system comprising two or more mixing units, a method for mixing two or more fluids and a lysis system.

[0002] Plasmid DNA (pDNA) represents a critical starting point for many genetic engineering pursuits, including the development of recombinant proteins, viral vectors, and advanced biotherapeutics. Plasmid-DNA (pDNA) is used as vaccines, gene therapy vectors or precursors for the production of therapeutic viral vectors and mRNA vaccines. Continued progress in gene therapy and DNA/RNA-based therapeutics has led to a growing demand for reliable pDNA production processes suitable for clinical applications.

[0003] Isolation of target pDNA requires removal of bacterial host-cell contaminants, including DNA, RNA, and host cell proteins (HCPs). Methods of isolation involve a (bacterial) cell lysis step in the presence of strong base (alkaline lysis), neutralization and precipitation to recover the extra-chromosomal sequence(s) in solution from precipitated host-cell contaminants. However, viscous solutions, accompanying lysis process, may cause local heterogeneities or require extensive mixing causing shear stress and degradation of pDNA. The problem increases with the volume/mass of cells, treated with a lysis solution.

[0004] Accordingly, there is a need for mixing methods and mixing devices particularly suitable for efficient mixing of feed streams comprising shear sensitive material and/or of different viscosities, as, e.g., encountered in pDNA isolation. The mixing method should be easily scaled-up and/or scaled-down, enabling the mixing of liquids in volume range from 0.1 up to 1000 L scale.

[0005] The most critical unit operation in large-scale pDNA lysis is the neutralisation reaction, where highly viscous and highly alkaline cellular lysate containing degraded cell components, including the product of interest, needs to be efficiently neutralized with minimal shear. The product of interest is sensitive to rapid degradation because the cell wall does not secure it. Most of the patents described above deal with improvements in this step.

[0006] The goal of efficient neutralization is to stop the alkaline lysis step, which is necessary to release the product of interest from the cytoplasm, at the point of maximum release and minimal degradation. This is generally achieved with efficient mixing systems, enabling mixing of solutions with large density differences, e.g. resuspended cell paste diluted in strong base, and low-pH buffer, under low shear and high speed of mixing. Literature describes the use of different liquid mixers including, but not limited to, in combination with gas.

[0007] It is therefore an object of the present invention to provide a mixing unit, a mixing system, a method for mixing and a lysis system for efficiently mixing components of a feed stream with low shear, in particular components having different viscosities.

Summary of the Invention

[0008] A first aspect of the present invention relates to a mixing unit for mixing two or more fluids, in particular fluids containing shear sensitive biological material, the mixing unit comprising: a first inlet for receiving an inlet stream, the inlet stream comprising a first fluid and a second fluid; a channel structure in fluid communication with the inlet, the channel structure comprising: a first branching for splitting the inlet stream into a first and a second primary branch streams (first and second primary flow paths); a first and a second primary channel, each configured for piping of one of respective primary branch streams, a first collecting reservoir for recombining the primary branch streams; wherein the first and second primary channels fluidly connect the first branching with the first collecting reservoir.

[0009] When the first fluid and the second fluid are combined, i.e., brought together, they are (often) not immediately forming a completely mixed, homogeneous fluid. Thus, the first inlet is configured to receive the inlet stream that comprises the inlet fluid which basically constitutes an incomplete/inhomogeneous mixture of the first and second fluid. In a mixing unit of the invention, the first branching is configured to split and/or divide the stream and/or the components of that inlet stream received through the first inlet into two or more separate branch streams. The branch streams may be considered as separate flow paths of the stream flowing through the mixing unit before they are recombined. The length of such a separate flow path (e.g., the shortest connection between the corresponding branching and the corresponding collecting reservoir) may be larger than the width of that flow path (e.g., at its narrowest point).

[0010] The separation of the fluid into at least two separate flow paths (e.g., the primary flow paths) may not yet achieve a full homogeneity in the mixture. Mixing is particularly achieved by splitting and/or dividing the inlet stream into at least two primary branch streams at a first branching together with recombining and/or reuniting the at least two primary branch streams in the first collecting reservoir.

[0011] At an outlet stage of the channels, the branch streams enter the first collecting reservoir and are reunited. Preferably, the position and/or orientation of the channels at the collecting reservoir cause collision of the branch streams, thereby improve mixing of the branch streams.

[0012] A respective mixing unit is advantageous for efficiently mixing the components of the inlet stream with low shear, in particular components and/or fluids of the inlet stream having different viscosities. Exemplary viscosities of the components and/or fluids of the inlet stream may be between approximately 1 and approximately 100 mPas.

[0013] The mixing unit may be configured to cause one or more collision(s) of parts of the inlet stream at or in one or more collecting reservoirs under an applied flow rate,

leading to very efficient mixing of the components of the inlet stream, in particular if said components have different and/or comparatively high viscosities.

[0014] The mixing unit may cause turbulent mixing in one or more sections of the channel structure, in particular in a mixing zone, which may correspond to a section of a collecting reservoir where the first branch stream and the second branch stream are reunited and/or collide with one another. Said mixing zone may comprise a section of a collecting reservoir with the smallest diameter and/or cross-section orthogonal to the flow direction of the stream.

[0015] Shear sensitive biological material may particularly comprise one or more of: active ingredient derived from cells such as pDNA, RNA, proteins, viruses, virus-like particles, extracellular vesicles (eVs) and/or their components.

[0016] As an example, in particular for pDNA isolation, the first fluid may comprise lysed cells containing DNA and/or other host cell contaminants (cell debris) as a first ingredient and a second ingredient comprising or being a lysis buffer (e.g., containing sodium hydroxide and/or sodium dodecyl sulphate) forming a basic (alkaline) environment.

[0017] As an example, in particular for pDNA isolation, the second fluid may comprise or be a neutralization agent and/or a neutralization buffer such as potassium acetate (K-acetate).

[0018] The inlet stream may comprise a further component, in particular a gaseous fluid such as gas, e.g., air and/or carbon dioxide. Bubbles of gas, in particular air, may enable more efficient mixing between the first fluid and the second fluid of the inlet stream.

[0019] The channel structure may be configured to pipe the inlet stream and/or mix the first fluid and the second fluid. The fluids being piped by the channel structure and originating from the inlet stream are described as stream in the following.

[0020] The mixing unit provides an advantageous low shear mixing geometry, which achieves rapid and mild mixing between the components of the inlet stream.

[0021] The channel structure may further comprise: a second branching for splitting the stream in the first collecting reservoir into a first and a second secondary branch streams; a first and a second secondary channel, each configured for piping of one of respective secondary branch streams; a second collecting reservoir for recombining the secondary branch streams; wherein the first and second secondary channels fluidly connect the second branching with the second collecting reservoir.

[0022] A respective mixing provides an improved mixing result due to the additional splitting and recombining of the stream.

[0023] The primary channels and the secondary channels may be arranged in a common plane. This way, a compact mixing unit body in combination with efficient mixing may be obtained. Alternatively or in addition, the distance the stream flows within the mixing unit may be reduced to decrease residence time, thereby improving throughput performance. A respective positioning avoids additional bends and/or curves which would cause undesired shear forces to the components of the stream.

[0024] The first branching, the first collecting reservoir, the second branching and the second collecting reservoir may be positioned on a common axis. The common axis may be substantially identical or parallel to longitudinal axis of the mixing unit. A respective positioning avoids additional and/or unnecessary bends and/or curves causing shear forces acting on the components of the stream. Thus, a respective positioning may improve low shear piping of the stream and/or mixing of the components of the stream.

[0025] The first inlet and the outlet of the mixing unit may be positioned on said common axis.

[0026] The mixing unit may improve mixing efficiency through an increased number of collision and/or recombination points of the stream. The channel structure may be configured to split and recombine the stream multiple times, e.g., two, three, four, five, six or more times. A higher number of splitting and recombining may improve the obtained mixing result.

[0027] In particular, the channel structure may further comprise a third branching for splitting the stream in the second collecting reservoir into a first and a second tertiary branch streams; a first and a second tertiary channel, each configured for piping of one of respective third branch streams; a third collecting reservoir for recombining the tertiary branch streams; wherein the first and second tertiary channels fluidly connect the third branching with the third collecting reservoir.

[0028] The properties described with respect to the primary and/or secondary channels may be accordingly directed to the tertiary, quaternary, quinary, senary, ... channels. The same applies with respect to the first and/or second branching(s) and/or the first and/or second collecting reservoir(s).

[0029] The mixing unit may be configured to: achieve low shear mixing of the components of the inlet stream; and/or mix the components of the inlet stream at a low Reynolds number, preferably at a Reynolds number between approximately 6000 and approximately 70000, in particular between approximately 10000 and 30000. Low shear mixing and/or mixing at a low Reynolds number avoids or at least reduces degradation of the biological material of interest and, therefore, improves process performance.

[0030] The mixing unit is preferably configured to mix the components of the (inlet) stream at the above-stated Reynolds numbers with one or more of the process characteristics and/or properties of the mixing method according to a fourth aspect of the present invention specified further below.

[0031] The volumes of the first and second primary channels are substantially identical; and/or wherein the volumes of the first and second secondary channels are substantially identical. The stream may be split into sub-

stantially identical parts and/or volumes by the first branching. Accordingly, substantially equal mixing of all branch streams may be achieved. Alternatively or in addition, substantially identical residence time and/or flow rate of the parts of the stream in the different channels may be obtained.

[0032] It is preferred that the flow resistance of all primary channels and/or of all secondary channels and/or of all tertiary channels are substantially identical so that an equal distribution of the respective streams is obtained.

[0033] An entrance angle between the main flow directions of the branch streams at respective entrance sections of the first and second channels (primary and/or secondary channel) at the associated branchings is substantially between approximately 40° and approximately 120°, preferably between approximately 70° and approximately 90°, and, preferably, is oriented symmetrically with respect to a longitudinal axis of the mixing unit. The entrance angle is more exemplary shown in and described with reference to Fig. 2 of the application further below.

[0034] The larger the entrance angle formed by the entrance sections of neighbouring primary and/or secondary channels, the higher the shear forces acting on the components of the stream are due to the splitting, in particular caused by a high change in flow direction as compared to the flow direction in the corresponding collecting reservoir. The lower the entrance angle formed by the entrance sections of the primary and/or secondary channels, the smaller the shear forces acting on the components of the stream are due to the splitting, in particular caused by a low change in flow direction as compared to the flow direction in the corresponding collecting reservoir.

[0035] As an example, an angle of between approximately 70° and approximately 90°, e.g., approximately 80°, is appropriate for efficient mixing while ensuring an appropriate flow rate and/or residence time in the mixing unit, in particular for fluids having different viscosities.

[0036] One or more of the branchings may be formed by a wall portion of the channel structure comprising a substantially rounded shape oriented towards the mixing zone and/or substantially against the flow direction of the stream in the collecting reservoir at or directly after collision of the branch streams. This provides a decent compromise between improving mixing and decreasing shear forces. Alternatively, one or more of the branchings may be formed by protuberance of a wall portion of the channel structure extending in a direction against the flow direction of the stream in the collecting reservoir at or directly after collision of the branch streams, optionally with an apex positioned at a center and/or at or parallel to a longitudinal axis of the mixing unit. This provides weaker mixing performance but significantly reduces shear forces due to a relatively gently guiding and/or splitting of the stream. Alternatively, one or more of the branchings may be formed by a wall portion of the channel structure comprising a substantially flat shape oriented substantially orthogonal with respect to the flow direction of the stream in the collecting reservoir at or directly after collision of the branch streams. This provides high mixing performance but with significantly high shear forces due to a head-on collision of the stream.

[0037] An exit angle between the main flow direction of the branch stream at an exit section of the first and second channels (primary and/or secondary) at the associated collecting reservoir is substantially between approximately 40° and approximately 120°, preferably between approximately 70° and approximately 90°, and, optionally, is oriented symmetrically with respect to a longitudinal axis of the mixing unit. The exit angle is more exemplary shown in and described with reference to Fig. 2 of the application further below.

[0038] The larger the exit angle formed by the exit sections of the primary and/or secondary channels, the higher the shear forces acting on the components of the fluids due to the higher relative velocity of the branch streams prior to the collision, which causes improved mixing but increases shear forces acting on the components of the stream.

[0039] As an example, an angle of between approximately 70° and approximately 90°, e.g., approximately 80°, is advantageous for ensuring an efficient and low shear mixing, in particular for fluids having different viscosities.

[0040] The entrance angle and the exit angle of the first and second primary branch streams may be substantially identical or different.

[0041] The primary and/or secondary channels may comprise one or more sections having a substantially circular and/or oval cross-sectional shape substantially orthogonal to the flow direction of the respective branch stream. In particular, it is preferred that substantially no sharp edges are present in the channels, which would cause increased shear forces acting on the components of the (branch) stream. However, different shapes are possible, such as substantially rectangular or substantially kidney-shaped.

[0042] The channels may comprise a reduced diameter portion with a reduced cross-section substantially orthogonal to a flow direction of the stream compared to an average cross-section of the respective channel. Preferably the reduced diameter portion is at a position closer to the associated collecting reservoir than to the associated branching and/or at or near an exit section of the channel. The flow velocity of the branch stream may be increased at and/or in the reduced diameter portion so that collision at higher velocities is obtained for more efficient mixing, in particular for fluids having higher viscosities.

[0043] A ratio between the cross-section (e.g., cross sectional area) of the reduced diameter portion and the average cross-section of a channel may be between approximately 1:1 to approximately 1:12, in particular between approximately 1:3 to approximately 1:7.

[0044] The first and second primary channels may be substantially congruent, so that mixing properties of all primary channels are substantially identical and that higher homogeneity of the stream may be obtained. Congruent may relate to substantially the same size and/or substantially the same shape.

[0045] The first and second secondary channels may be substantially congruent, so that mixing properties of all secondary channels are substantially identical and that higher homogeneity of the stream may be obtained.

[0046] All of the primary channels and/or all of the secondary channels may be substantially congruent. Specifically, if the first branching and the first collecting reservoir are connected via multiple (separate) channels (i.e., primary channels), such as three, four, five, or more, they may be substantially congruent to each other. This may also apply to multiple secondary channels with respect to each other. Moreover, even the primary channels and the secondary channels may be substantially congruent to each other.

[0047] However, it may be beneficial to adapt the secondary channels and/or further subsequent channels to the mixing degree and/or degree of homogeneity of the stream. For example, the secondary channels and/or the tertiary channels may have a different shape and/or size than the primary channels because the degree of mixing in the stream flowing through the secondary channels and/or the tertiary channels is higher than that of the stream flowing through the primary channels. Accordingly, efficient mixing may be obtained even with reducing shear forces acting on the components of the stream at collision(s) in the second and/or third collecting reservoir, e.g., by reducing an exit angle and/or flow velocity of the branch streams.

[0048] The inlet stream may be split into substantially identical parts and/or volumes by the first branching. Accordingly, substantial equal mixing of all branch streams may be achieved.

[0049] The primary channels may be positioned substantially symmetrically with respect to a longitudinal axis of the mixing unit; and/or the secondary channels may be positioned substantially symmetrically with respect to a longitudinal axis of the mixing unit. A respective positioning may avoid additional and/or unnecessary bends and/or curves, which would cause undesired shear forces to the components of the stream.

[0050] The mixing unit may further comprise a second inlet provided at one of the collecting reservoirs for introducing a third fluid into the respective collecting reservoir.

[0051] In an example, in particular for pDNA isolation, the third fluid may be or comprise a precipitation agent such as CaCl2 to improve pDNA separation.

[0052] Preferably, the second inlet is provided at a collecting reservoir having a substantially equal number of preceding and subsequent branchings and/or collecting reservoirs.

[0053] Accordingly, a substantial equal mixing of the components of the inlet stream prior to the second inlet and of the stream with the added third fluid after the second inlet may be achieved.

[0054] The mixing unit may be manufactured using 3D printing technology. This allows a customized and/or customizable design and/or one-piece manufacturing of the mixing unit.

[0055] The mixing unit may be 3D printed from a biocompatible printing material, e.g., PA2200.

[0056] The mixing unit may comprise a coating including inert polymer, e.g., Parylene C. In particular the channel structure and/or inner surfaces coming into contact with the fluids may be coated with an inert polymer.

[0057] A second aspect of the present invention relates to a use of the mixing unit according to a first aspect for substantially continuous processing of one or more of: cell lysis, neutralization, clarification, nucleic acid purification and concentration, wherein a more efficient process may be obtained, in particular compared to batch processing.

[0058] A third aspect of the present invention relates to a mixing system for mixing two or more fluids, the mixing system comprising: a first mixing unit according to the first aspect; and a second mixing unit according to the first aspect; wherein the first mixing unit and the second mixing unit are fluidly connected in series wherein the first inlet of the second mixing unit is fluidly connected to an outlet of the first mixing unit.

[0059] The mixing system may be considered as modular mixing system, wherein two or more mixing units, each comprising a separate and/or separable body, are fluidly connected to one another so that the stream consecutively passes through each one of the mixing units.

[0060] For example, a mixing unit may comprise three, four, five or more mixing units.

[0061] One or more of the mixing units may be oriented to achieve a bottom-up and/or bottom to top fluid flow direction of the stream within the mixing unit. A bottom-up fluid flow direction may increase mixing result, in particular at low flow rates of the stream.

[0062] One or more of the mixing units of a mixing system may be positioned substantially along a common axis. Alternatively or in addition, one or more of the mixing units of a mixing system may be positioned substantially parallel to one another.

[0063] Preferably, the mixing units have a common orientation, e.g., with their respective first inlets at a bottom position and their respective outlets at a top position.

[0064] One or more mixing units may be oriented so that their respective first inlet is lower than an outlet of a preceding mixing unit, wherein a connection pipe and/or tube leads from the outlet of the preceding mixing unit to the first inlet of the subsequent mixing unit.

[0065] The mixing system may further comprise a connection part for fluidly connecting the outlet of the first mixing unit with the first inlet of the second mixing unit, wherein the connection part comprises a second inlet for

receiving a third fluid.

[0066] In an example, in particular for pDNA isolation, the third fluid may be or comprise a precipitation agent such as CaCl2 to improve pDNA separation.

[0067] The volume within the connection part may be at least a part of the last collecting reservoir of preceding first mixing unit.

[0068] A fourth aspect of the present invention relates to a method of mixing two or more fluids, in particular fluids containing shear sensitive biological material and/or their components, the method comprising the steps of: splitting an inlet stream comprising a first fluid and a second fluid into a plurality of primary branch streams; and recombining the plurality of primary branch streams in a first collecting reservoir.

[0069] By splitting the stream into two or more branch streams and by recombining and/or reuniting the two or more branch streams, efficient low shear mixing of the components of the stream may be obtained.

[0070] The method may further comprise the steps of: splitting the stream in the first collecting reservoir into a plurality of secondary branch streams; and recombining the plurality of secondary branch streams in a second collecting reservoir.

[0071] With additional splittings and recombinations, the mixing of the components of the stream and/or homogeneity of the stream may be improved.

[0072] The method may further comprise splitting the stream in the second collecting reservoir into a plurality of tertiary branch streams and recombining the plurality of tertiary branch streams in a third collecting reservoir.

[0073] The method may further comprise splitting the stream in the third collecting reservoir into a plurality of quaternary branch streams and recombining the plurality of quaternary branch streams in a fourth collecting reservoir.

[0074] The method may further comprise splitting the stream in the fourth collecting reservoir into a plurality of quinary branch streams and recombining the plurality of quinary branch streams in a fifth collecting reservoir.

[0075] The components of the inlet stream may be mixed at low shear and/or the components of the inlet stream may be mixed at a low Reynolds number, preferably at a Reynolds number between approximately 6000 and approximately 70000, in particular between approximately 10000 and 30000.

[0076] A time of the stream between a first splitting and a final recombining may be between approximately 0.1 seconds and approximately 30 seconds, preferably between approximately 1 seconds and approximately 15 seconds.

[0077] A flow rate of the stream may be between approximately 50 mL/min and approximately 20 L/min, preferably between approximately 150 mL/min and approximately 2000 mL/min.

[0078] An average flow velocity of the stream may be between approximately 0.03 m/s to approximately 0.4 m/s, in particular in a region of the collecting reservoir where the branch streams are recombined and/or collide.

[0079] The average flow velocity of a branch stream may be between approximately 0.03 m/s to approximately 0.4 m/s, in particular at a section of a channel having the smallest cross-sectional area and/or at the reduced diameter portion.

[0080] The method may include piping and/or directing the stream and/or branch streams in correspondence to what is described with respect to other aspects of the present invention, in particular the first aspect of the present invention.

[0081] The mixing unit according to the first aspect is preferably configured to mix the components of the (inlet) stream at the above-stated Reynolds numbers, in particular with one or more of the process characteristics and/or properties laid out in the foregoing.

[0082] A fifth aspect of the present invention relates to a lysis system for lysing cells containing double stranded DNA, the lysing unit comprising: a mixing system according to the second aspect of the present invention or a mixing unit according to the first aspect of the present invention having a second inlet for introducing a precipitation agent to the mixed components of the inlet stream; a mixing chamber for mixing of the first fluid of the inlet stream comprising a lysis buffer and a fluid comprising resuspended cells; an incubation tube fluidly connected to an outlet of the mixing chamber for piping of the first fluid of the inlet stream during lysis reaction of the cells; and a first joint for joining the first fluid and the second fluid of the inlet stream, wherein the first joint is fluidly connected to the first inlet of the first mixing unit of the mixing system respectively to the mixing unit.

[0083] The first joint may be provided for pre-mixing of components of inlet stream before entering the mixing first mixing unit.

[0084] The mixing chamber and the incubation tube may be configured to hold and/or pipe the first fluid while cell lysis occurs. The mixing chamber may comprise a helical static mixer and/or the incubation tube may comprise a coil.

[0085] Preferably, the mixing chamber and/or the incubation tube provide a bottom-up flow direction and/or a flow rate of approximately 40 mL/min to approximately 400 mL/min A residence time of the ingredients of the first fluid in the mixing chamber may be between approximately 1 to approximately 10 seconds, in particular between approximately 2 seconds to approximately 8 seconds and/or a residence time of the ingredients of the first fluid in the incubation tube may be between approximately 1 minute and approximately 15 minutes, in particular between approximately 3 minutes and approximately 10 minutes. The incubation tube may comprise a volume of between approximately 500 mL and approximately 2000 mL, e.g., approximately 1000 mL, and/or may have a tube diameter of between approximately 0.3 cm and approximately 1.5 cm, e.g., approximately 0.6cm.

[0086] The lysis system may further comprise a third inlet for introducing a fourth fluid, in particular gaseous

fluid such as air to the first and second fluids of the inlet stream.

[0087] This may enable more efficient mixing between the first fluid and the second fluid of the inlet stream.

[0088] The lysing system may further comprise one or more pumps for pumping a fluid and/or one or more vessels for holding a fluid and/or one or more, preferably contactless, flow pressure and/or flow rate sensors and/or one or more pH control units and/or one or more temperature control units. The allows an efficient implementation of the invention in (or its use in connection with) filtration and chromatography, e.g. after the lysis in an automated way using such a lysis system.

[0089] A sixth aspect of the present invention relates to a method for lysing cells containing double stranded DNA comprising the steps of: mixing a fluid stream comprising: a first fluid comprising resuspended cells and a lysis buffer; and a second fluid comprising a neutralization buffer in a first mixing unit; adding an auxiliary fluid (that may also be considered a third fluid herein) comprising a precipitation agent to the mixed first fluid and second fluid; and mixing of the first fluid, the second fluid and the auxiliary (i.e. third) fluid in the first mixing unit or in a second mixing unit.

[0090] Alternatively or additionally, the method may further comprise introducing a mixing support fluid (that may also be considered a fourth fluid herein), in particular a gaseous fluid such as air, to the first fluid and the second fluid of the fluid stream, optionally, at a flow rate of between approximately 0.5 L/min and approximately 10 L/min.

[0091] This may enable more efficient mixing between and more homogenization of the first fluid and the second fluid in the inlet stream. By introducing gas, higher yield and higher purity of the product of interest may be obtained. The mixing support fluid (even when considered as "fourth" fluid herein) may be added before (i.e. at a position upstream to) adding the auxiliary fluid (even when considered as "third" fluid herein).

[0092] The lysis system according to the fifth aspect and/or the method according to the sixth aspect are particularly advantageous for substantially continuous processing of cell lysis. Therefore, no need for harvesting the obtained produce is required.

[0093] In particular, they provide advantages, in particular over batch lysis, including higher control (better control over the lysis reaction) and consequently the robustness of the lysis as well as easy scale-up and/or the possibility of processing a biomolecule to the point where it is no longer subject to rapid degradation. Also, separate mixing steps may be performed timely consecutively so that improved integrity and/or less degradation of target product may be obtained.

[0094] The present invention is further explained in detail by the following detailed description and the appended drawings, in which particular embodiments are illustrated by way of example, wherein the present invention is in no way limited by these particular embodiments.

Brief description of the drawings

[0095]

Fig. 1	shows an exemplary mixing unit;
Fig. 2	shows a schematic of the channel structure of the exemplary mixing unit of Fig. 1;
Fig. 3	shows a cross-sectional view of the exemplary mixing unit of Fig. 1;
Fig. 4a-d	show cross-sectional views of the exemplary mixing unit at the correspondingly labeled lines shown in Fig. 3;
Fig. 5	shows a simulation of an exemplary flow pattern of a stream flowing through the channel structure;
Fig. 6	shows a simulation of exemplary flow velocities of a stream flowing through a part of the channel structure;
Fig. 7	shows an exemplary lysis system;
Fig. 8	shows an exemplary mixing system comprising two exemplary mixing units
Fig. 9	shows another exemplary mixing unit.
Fig. 10	shows analytical HPLC chromatograms (CIMac pDNA column) comparing product quality and quantity of 4.7 kbp plasmid isolated from *E. coli* using batch lysis (1g of E. *coli* cell paste; full line) and in-line lysis (100 g E. *coli* cell paste; dashed line).

Description of particular embodiments

[0096] **Fig. 1** shows an exemplary mixing unit 1 for mixing two or more fluids of an inlet stream that enters via a first inlet 4.

[0097] The shown exemplary mixing unit 1 comprises an elongated and/or relatively flat body 2, which houses a channel structure for piping the inlet stream.

[0098] The mixing unit 1 comprises the first inlet 4, which may be fluidly coupled to a pipe and/or a hose, and/or which is configured to receive the inlet stream.

[0099] The inlet stream may comprises two or more components, in particular at least a first fluid and a second fluid, which may be pre-mixed in the inlet stream and/or substantially simultaneously entering the mixing unit 1 through the first inlet 4.

[0100] The mixing unit 1 comprises an outlet 6 through which the stream at least partly originating from the inlet stream exits the body 2 of the mixing unit 1, preferably in a substantially mixed state and/or with a high degree of mixing and/or homogeneity.

[0101] In the shown exemplary mixing unit 1, the first inlet 4 and the outlet 6 are substantially positioned along a common axis, which is substantially identical or parallel with a longitudinal axis 3 of the body 2 of the mixing unit 1. This allows a compact size of the mixing unit 1 and/or a small flow distance between the first inlet 4 and the outlet

6.

[0102] Optionally, the body 2 of the mixing unit 1 may comprise one or more openings and/or recesses, in particular in order to reduce material and/or weight of the mixing unit 1. The shown example comprises seven substantially circular openings fully extending through the body 2.

[0103] **Fig 2** shows a schematic of the channel structure of the exemplary mixing unit 1 of Fig. 1.

[0104] The channel structure connects the first inlet 4 with the outlet 6 of the mixing unit 1. The channel structure provides one or more flow paths for piping the inlet stream 5 received at the first inlet 4 towards the outlet 6.

[0105] The exemplary mixing unit 1 comprises a channel structure, which may divide and recombine the stream multiple times, specifically three times.

[0106] Along the flow direction of the inlet stream 5, the channel structure comprises a first branching 10, which is configured to direct the inlet stream 5 into two primary channels 14-1, 14-2. The first branching 10 is configured to divide and/or split the inlet stream 5 into a first primary branch stream 12-1 piped by a first primary channel 14-1 and into a second primary branch stream 12-2 piped by a second primary channel 14-2.

[0107] In the shown example, the first primary channel 14-1 and the second primary channel 14-2 are arranged substantially in a common plane and/or substantially symmetrically, specifically relative to a longitudinal axis 3 of the body 2 of the mixing unit 1.

[0108] The channel structure may comprise further primary channels dividing the inlet stream 5 into a corresponding number of branch streams. In this case, the primary channels may be substantially radially oriented and/or arranged along respective planes intersecting in a common axis, in particular the longitudinal axis 3 of the body 2 of the mixing unit 1. In other words, if the channel structure comprises three or more primary channels, the primary channels may be oriented radially and/or star-shaped when viewed along a longitudinal axis 3 of the body 2 of the mixing unit 1.

[0109] Preferably, the angles between neighboring channels and/or their respective planes, are substantially identical.

[0110] Alternatively or in addition, at least opposing angles may be substantially identical. This way, a more compact body 2 may be obtained while still achieving a high uniformity of the branch streams.

[0111] Preferably, the volumes and/or sizes and/or shapes of all primary channels 14-1, 14-2 are substantially identical.

[0112] A respective branching 10 may be configured to divide and/or split the inlet stream 5 into substantially identical parts. Thus, the first primary branch stream 12-1 and the second primary branch stream 12-2 are configured to pipe substantially identical volumes of the stream and/or at substantially identical flow rates.

[0113] The primary branch streams 12-1 and 12-2 are configured to respectively pipe and/or guide the primary branch streams 12-1 and 12-2 to a first collecting reservoir 18. The first collecting reservoir 18 is configured to receive all of the primary branch streams 12-1, 12-2.

[0114] Preferably the inlets through which the primary branch streams 12-1, 12-2 enter the first collecting reservoir 18 are positioned and/or orientated relative to one another in such a way that the first primary branch stream 12-1 and the second primary branch stream 12-2 upon entering the first collecting reservoir 18 collide with one another and are subject to turbulent mixing and/or laminar mixing.

[0115] The colliding of branch streams achievable by the exemplary mixing unit 1 corresponds to a particular advantageous mixing of the components of the stream with high efficiency and low shear. Accordingly, high homogeneity of the stream may be achieved while reliably preserving shear-sensitive material comprised by the stream.

[0116] The exemplary mixing unit 1 further comprises a second branching 20, which divides and/or splits the stream in the first collecting reservoir 18 into two secondary branch streams 22-1, 22-2. The second branching 20 may comprise substantially identical properties and/or functions as described with respect to the first branching 10.

[0117] In particular, the second branching 20 is configured to direct the stream into two or more secondary channels, such as first secondary channel 24-1 and second secondary channel 24-2.

[0118] The secondary channels 24-1, 24-2 are configured to pipe and/or guide respective secondary branch streams 22-1, 22-2 to a second collecting reservoir 28, in which the secondary branch streams 22-1, 22-2 are mixed by colliding with each other. Accordingly, due to the additional splitting and recombining, the degree of mixing of the components of the stream may be increased.

[0119] The same applies for the third branching 30, which is configured to divide the stream in the second collecting reservoir 28 into a plurality of tertiary branch streams, in the shown example into a first tertiary branch stream 32-1 and a second tertiary branch stream 32-2 respectively piped by a first tertiary channel 34-1 and a second tertiary channel 34-2, and recombining the same in a third collecting reservoir 38, thereby further increasing the degree of mixing and/or homogeneity of the stream.

[0120] In the exemplary mixing unit 1, the third collecting reservoir 38 is positioned directly upstream of the outlet 6 through which the mixed stream exits the mixing unit 1. However, in other examples, further mixing stages fluidly coupled to one another in series between the third collecting reservoir 38 and the outlet 6 may be comprised by the mixing unit 1. Each of said mixing stages preferably comprising a branching, two or more channels and a collecting reservoir, wherein each stage may improve the degree of mixing and/or homogeneity of the stream.

[0121] An entrance angle 15a of entrance sections 15

of the primary channels 14-1, 14-2 may be between approximately 40° and approximately 120°, preferably between approximately 70° and approximately 90°, for example approximately 80°. The entrance angle 15a may substantially define the angle in which the primary channels 14-1, 14-2, in particular entrance sections 15 of the primary channels 14-1, 14-2 located close and/or directly adjacent to the first branching 10, are oriented relative to one another.

[0122] An entrance section 15 may for example comprise or constitute a first section of the channel at which the branch streams enter the channel. For determining the entrance angle 15, an average flow direction of the branch streams 12-1, 12-2 flowing through the respective entrance sections 15 of the channels 14-1, 14-2 is determined by approximation and the angle enclosed by the determined flow directions provides a measure of the entrance angle 15a.

[0123] A larger entrance angle 15a may provide a more efficient mixing, while at the same time increasing shear forces acting on the components of the stream due to a higher degree of redirection of the stream. A smaller angle may provide a less efficient mixing, while at the same time decreasing shear forces acting on the components of the stream due to a smaller degree of redirection of the stream.

[0124] An exit angle 16a of exit sections 16 of the primary channels 14-1, 14-2 may be between approximately 40° and approximately 120°, preferably between approximately 70° and approximately 90°, for example approximately 80°. The exit angle 16a may substantially define the angle in which the primary channels 14-1, 14-2, in particular exit sections 16 of the channels 14-1, 14-2 located close and/or directly adjacent to the first collecting reservoir 18, are oriented relative to one another.

[0125] An exit section 16 may for example comprise or constitute a last section of the channel which the branch streams flow through immediately before exiting the channels and/or entering the first collecting reservoir 18. For determining the exit angle 16, an average flow direction of the branch streams 12-1, 12-2 flowing through respective the exit sections 16 of the channels 14-1, 14-2 is determined by approximation and the angle enclosed by the determined flow directions provides a measure of the exit angle 16a.

[0126] The exit angle 16a may further describe the angle at which collision occurs between the branch streams 12-1, 12-2 in the first collecting reservoir 18.

[0127] A larger exit angle 16a may provide a more efficient mixing, while at the same time increasing shear forces acting on the components of the stream caused by a higher relative velocity of the branching streams. A smaller angle may provide a less efficient mixing, while at the same time decreasing shear forces acting on the components of the stream due to a lower relative velocity of the branching streams.

[0128] The primary channels 14-1, 14-2 may each comprise a reduced diameter portion 17 with a reduced cross-sectional area substantially orthogonal to a flow direction of the respective branch streams 12-1, 12-2 compared to an average cross-sectional area of the respective channel. Preferably the reduced diameter portions 17 of the primary channels 12-1, 12-2 are at a position closer to the first collecting reservoir 18 than to the first branching 10 and/or at or near an exit section 16 of the channels. The flow velocity of the branch streams 12-1, 12-2 may be increased at and/or in and/or after the reduced diameter portion 17 so that collision at higher velocities of the branch streams 12-1, 12-2 may be obtained. This way, a more efficient mixing of the components of the stream may be achieved, in particular for fluids having higher viscosities.

[0129] A ratio between the cross-section of the reduced diameter portion 17 and the average cross-section of a channel may be between approximately 1:1 and approximately 1:12, in particular between approximately 1:3 and approximately 1:7.

[0130] The channel structure may have a volume between approximately 10 mL and approximately 1000 mL, in particular between approximately 20 mL and approximately 100 mL. In other words, the mixing unit 1 may be configured to hold and/or receive between approximately 10 mL and approximately 1000 mL, in particular between approximately 20 mL and approximately 100 mL, of fluid(s).

[0131] **Fig. 3** shows a cross-sectional view of the exemplary mixing unit 1 of Fig. 1 wherein the intersecting plane is oriented substantially parallel to a longitudinal axis 3 of the body 2 of the mixing unit 1 and the intersecting plane substantially bisects the channel structure and the first inlet 4 and the outlet 6 of the mixing unit 1.

[0132] The channel structure of the exemplary mixing unit 1 comprises substantially rounded and/or beveled corners and/or edges at the channels and the collecting reservoirs. This is advantageous as it reduces shear acting on the components of the stream, in particular at bends and/or curves, compared to sharp edges. The same applies for the branchings. In addition, volume of dead spaces and/or spaces in which low flow velocities occur may be reduced. Also, a more uniform velocity distribution throughout a cross-section of the stream may be obtained.

[0133] Preferably, at least some of the channels 14-1, 14-2, 24-1, 24-2, 34-1, 34-2, the collecting reservoirs 18, 28, 38 and/or the branchings 10, 20, 30 are arranged substantially on a common level with respect to a longitudinal axis 3 of the body 2. By this, a substantially uniform flow of the stream through the mixing unit 1 may be achieved because no bends apart from the channels exist.

[0134] **Fig. 4a-d** show cross-sectional views of the exemplary mixing unit 1 at the correspondingly labeled lines shown in Fig. 3.

[0135] Fig. 4a shows a cross-sectional view at an intersecting plane oriented substantially orthogonal to

a longitudinal axis 3 of the body 2 and positioned at line a-a shown in Fig. 3.

[0136] The first and second primary channels 14-1, 14-2 are intersected substantially at a position in the body 2, where a distance in a lateral direction of the body 2 is maximal. Said position may comprise a turning point of the primary channels 14-1, 14-2 between the first branching 10 and the first collecting reservoir 18, in which the flow direction is substantially parallel to the longitudinal axis 3 of the body 2.

[0137] At the intersected position shown in Fig. 4a, the first primary branch stream 12-1 and/or the second primary branch stream 12-2 have a flow direction substantially orthogonal to the intersecting plane.

[0138] In the shown example, the first and second primary channels 12-1 and 12-2 comprise a substantially oval cross-section and/or a cross-sectional area of between approximately 50 mm^2 and approximately 400 m^2, for example approximately 200 mm^2.

[0139] Fig. 4b shows a cross-sectional view at an intersecting plane oriented substantially orthogonal to a longitudinal axis 3 of the body 2 and positioned at line b-b- shown in Fig. 3.

[0140] In the shown example, the reduced diameter portions 17 is positioned at the exit sections 16 of the channels 14-1, 14-2.

[0141] The first and second primary channels 14-1, 14-2 are intersected at the reduced diameter portions 17, where a cross-sectional area of the channels 14-1, 14-2 is minimal.

[0142] However, at the intersected position shown in Fig. 4b, the first primary branch stream 12-1 and the second primary branch stream 12-2 have a flow direction not orthogonal to the intersecting plane and/or a flow direction towards one another. Accordingly, Fig. 4b does not illustrate the actual cross-sectional area orthogonal to the flow direction of the branch streams 12-1, 12-2, which is smaller than the oval opening illustrated in Fig. 4b.

[0143] In the shown example, the reduced diameter portions 17 of the first and second primary channels 12-1 and 12-2 comprise a substantially oval cross-section and/or a cross-sectional area orthogonal to the flow direction of the branch streams 12-1, 12-2 of between approximately 10 mm^2 and approximately 100 m^2, for example approximately 30 mm^2.

[0144] A ratio between the cross-sectional area at the reduced diameter portion and an average cross-section area of a channel may be between approximately 1:1 and approximately 1:10.

[0145] Fig. 4c shows a cross-sectional view at an intersecting plane oriented substantially orthogonal to a longitudinal axis 3 of the body 2 and positioned at line c-c- shown in Fig. 3.

[0146] The first collecting reservoir 18 is intersected substantially at a position in the body 2, where the primary branch streams 12-1, 12-2 collide with one another. In the shown example, this position substantially corresponds to the part of the first collecting reservoir 18 having the smallest cross-sectional area.

[0147] At the shown intersected position, an average flow direction of the stream is substantially identical or parallel with the longitudinal axis of the body 2 and/or substantially orthogonal to the intersecting plane.

[0148] In the shown example, the intersected part of the first collecting reservoir 18 may have a cross-sectional area of between approximately 20 mm^2 and approximately 150 m^2, for example approximately 50 mm^2.

[0149] Fig. 4d shows a cross-sectional view at an intersecting plane oriented substantially orthogonal to a longitudinal axis 3 of the body 2 and positioned at line d-d- shown in Fig. 3.

[0150] The first collecting reservoir 18 is intersected substantially at a position immediately upstream of the second branching 20, where the first collecting reservoir 18 merges into the secondary branch streams 22-1, 22-2. In the shown example, this position substantially corresponds to the part of the first collecting reservoir 18 having the largest cross-sectional area.

[0151] At the shown intersected position, the stream is divided and diverted towards the first and the second secondary channels 24-1, 24-2. Said position may substantially correspond to a position where the highest shear forces are acting on components of the stream due to the relatively strong redirecting of the stream caused by the branching.

[0152] In the shown example, the intersected part of the first collecting reservoir 18 may have a cross-sectional area of between approximately 100 mm^2 and approximately 1000 m^2, for example approximately 500 mm^2.

[0153] **Fig. 5** shows a simulation of an exemplary flow pattern of a stream flowing through the channel structure of the exemplary mixing unit of Fig. 1.

[0154] Pathlines and/or field lines indicate the direction and velocity of the stream flowing through the channel structure, wherein darker areas indicate a higher velocity and/or laminar flow of the stream and brighter areas indicate a lower velocity and/or a turbulent flow of the stream.

[0155] As indicated, the inlet stream 5 is redirected at the first branching 10 and divided into first and second primary branch streams 12-1, 12-2. The velocity of the stream(s) is higher close to a wall portion of the channel structure against which the part of the inlet stream having a high velocity clashes.

[0156] As further indicated, the distribution of flow velocity across the primary branch streams 12-1, 12-2 equalizes in a middle section of the primary channels 14-1, 14-2.

[0157] Upon reaching the reduced diameter portions 17, the flow velocity increases due to the reduced cross-sectional area through which the branch stream 12-1, 12-2 is forced.

[0158] The branch streams 12-1, 12-2 collide at high velocities with one another in the first collecting reservoir 18 and clash against the second branching 20. By this,

advantageous low shear mixing of the components of the stream is achieved.

[0159] The flow pattern substantially identically duplicates in the further mixing stages of the secondary and tertiary channels 22-1, 22-2, 32-1, 32-2.

[0160] **Fig. 6** shows a simulation of exemplary flow velocities of a stream flowing through a part of the channel structure of a section of the exemplary mixing unit 1 of Fig. 1.

[0161] The stream comprises a shaded indication of flow velocities, wherein darker areas correspond to areas of higher flow velocity and brighter areas correspond to areas of lower flow velocity.

[0162] A scale ranging from 0.0 m/s to 0.4 m/s is provided for a qualitative evaluation of the shown simulation.

[0163] A maximum velocity of approximately 0.25 m/s may be obtained in an area where the stream has been redirected by the branching 20.

[0164] A slightly lower velocity of approximately 0.2 m/s may be obtained at the area where the branch streams 12-1, 12-2 collide.

[0165] A lowest velocity of close to 0.0 m/s may be obtained in side areas of the first collecting reservoir 18 close to the point of collision of the branch streams 12-1, 12-2.

[0166] The simulation represents the following characteristics of the inlet stream and/or the channel structure: A cross-sectional area at the thinnest portion of the collecting reservoirs 18, 28, 38 has an oval shape and is approximately 50 mm^2. A cross-sectional area of the reduced diameter portion 17 has an oval shape and is approximately 100 mm^2. The entrance angle of the channels is approximately 80°. An exit angle of the channels and/or angle of collision of the branch streams is approximately 80°. The mixing unit comprises three mixing stages substantially oriented along a common axis. Viscosity of the first fluid of the inlet stream 5 is approximately 1 mPas and viscosity of the second fluid of the inlet stream 5 is approximately 2 mPas. Flow rates of the first liquid and the second liquid are substantially identical. A gas has been introduced to the inlet stream 5 at a flow rate of approximately 1 L/min at room temperature and standard atmospheric pressure.

[0167] **Fig 7** shows an exemplary lysis system 90 for lysing cells containing double stranded DNA. The lysis system 90 is particularly suitable for continuous processing of cell lysis and/or pDNA isolation.

[0168] The lysis system 90 may comprise a mixing system 80 including one or more mixing units 1 for mixing of at least a first fluid and a second fluid comprised by an inlet stream 5.

[0169] The lysis system 90 may further comprise one or more mixing chambers 92 for mixing of the first fluid of the inlet stream 5, respectively ingredients thereof. Each mixing chamber 92 may have a volume of between approximately 50 mL to approximately 500 mL.

[0170] The ingredients of the first fluid may comprise a fluid comprising resuspended cells, e.g., bacterial, algae, yeast and/or mammalian, and a lysis buffer, e.g., comprising sodium hydroxide and/or sodium dodecyl sulphate, for the purpose of lysing the resuspended cells.

[0171] The lysis system 90 may comprise a first tank 102 for storing and/or providing the lysis buffer to the mixing chamber 92. A first pump 103, in particular a peristaltic pump with a delivery rate of approximately 40 mL/min to approximately 400 mL/min, may be comprised by the lysis system 90 for delivering the lysis buffer from the first tank 102 to the mixing chamber 92.

[0172] The lysis system 90 may comprise a second tank 104 for storing and/or providing the fluid comprising resuspended cells to the mixing chamber 92. A second pump 105, in particular a peristaltic pump with a delivery rate of approximately 40 mL/min to approximately 400 mL/min, may be comprised by the lysis system 90 for delivering the fluid comprising resuspended cells from the second tank 104 to the mixing chamber 92.

[0173] A concentration of the resuspended cells in the fluid may be between approximately 0.1 g/mL and approximately 0.05 g/mL.

[0174] The lysis system 90 may further comprise an incubation tube 94 fluidly connected to an outlet of the mixing chamber 92 for piping of the first fluid of the inlet stream 5, respectively ingredients thereof, during lysis reaction of the cells. The incubation tube 94 may comprise a coil, e.g., with a diameter of between approximately 0.2 cm and approximately 2 cm, in particular approximately 0.6 cm. The incubation tube 94 may comprise a volume of approximately 1000 mL.

[0175] The mixing chamber 94 and the incubation tube 96 may be configured to hold and/or pipe the first fluid while cell lysis occurs.

[0176] The lysis system 90 may further comprise a first joint 96 for joining the first fluid subsequent to passing through the incubation tube 94 and the second fluid of the inlet stream.

[0177] The second fluid may comprise or be a neutralization agent and/or a neutralization buffer such as potassium acetate (3M K-acetate).

[0178] The lysis system 90 may comprise a third tank 106 storing and/or providing the second fluid to the first joint 96. A third pump 107, in particular a peristaltic pump with a delivery rate of approximately 40 mL/min to approximately 400 mL/min, may be comprised by the lysis system 90 for delivering the second fluid from the third tank 106 to the first joint 96.

[0179] The first joint 96 may be fluidly connected to a first inlet 6 of a mixing unit 1, in particular a first mixing unit 1 of the mixing system 80. The first joint 96 may be provided for pre-mixing of the components of inlet stream 5, in particular the first fluid and the second fluid, before entering the mixing unit 1.

[0180] Viscosity of the first fluid may be approximately 1 mPas and viscosity of the second fluid may be approximately 2 mPas or vice versa.

[0181] The first mixing unit 1 of the mixing system 80

may be according to an aspect herein and configured to mix the components of the inlet stream 5.

[0182] The mixing system 80 may further comprise a second inlet 84 for introducing an auxiliary fluid (which may also be called a "third" fluid herein), in particular a precipitation agent, such as CaCl2, to the inlet stream after being mixed by the first mixing unit 1.

[0183] The lysis system 90 may further comprise a third inlet 98 for introducing a fluid, in particular a gaseous fluid, such as gas and/or air, which may also be called a "fourth" fluid described herein, to the first fluid and the second fluid before entering the first mixing unit 1 for improving mixing efficiency. Therefore this "fourth" fluid may also be called mixing support fluid in this description. The gaseous fluid may be delivered at a flow rate of approximately 1 L/min and/or at room temperature and/or at standard atmospheric pressure. As shown in Fig. 7, the mixing support fluid (even when considered as "fourth" fluid herein) may be added before adding the auxiliary fluid and/or at a position upstream of the position the auxiliary fluid is added (even when considered as "third" fluid herein).

[0184] The lysis system 90 may comprise a fourth tank 108 for storing and/or providing the third fluid to the mixing system 80. A fourth pump 109, in particular a peristaltic pump with a delivery rate of approximately 40 mL/min to approximately 400 mL/min, may be comprised by the lysis system 90 for delivering the third fluid from the fourth tank 108 to the mixing system 80.

[0185] The second mixing unit 1 of the mixing system 80 may be according to an aspect herein and configured to mix the stream that at least partly originates from the inlet stream that has been mixed by the first mixing unit 1 and the third fluid introduced by the second inlet 84.

[0186] The lysing system 90 may comprise a collecting tank 100 for collecting the stream exiting the mixing system 80 and storing the same until further processing.

[0187] One or more flow rate sensors and/or pressure sensors, in particular contactless sensors, may be provided for process control, in particular between a pump and the mixing chamber 92 respectively the first joint 96 respectively the second inlet 82.

[0188] A method for continuous and in-line lysing of cells containing double stranded DNA, in particular using the lysing system 90, may comprise the following steps: Pumping resuspended cells and lysis buffer simultaneously with pumps 103, 105 and mixing the same in the mixing chamber 92 before piping through the incubation tube 96 (first fluid). The lysis step is neutralized at the first joint 96 by pumping a neutralization agent (second fluid), such as concentrated potassium acetate, joining it with the first fluid and mixing the first fluid and the second fluid in a first mixing unit 1. The neutralized solution is further mixed in a second mixing unit 1 where RNA impurities are precipitated by adding a precipitation agent, such as concentrated CaCl2, to the solution. The final lysate is collected in the collecting tank 100 and, e.g., ready for filtration.

[0189] **Fig. 8** shows an exemplary mixing system 80 comprising two mixing units 1, which may be comprised by a lysis system 90 according to an aspect herein.

[0190] The mixing system 80 may comprise a first mixing unit 1-1 and a second mixing unit 1-2 fluidly connected in series by a connecting part 82 connecting the first inlet 4 of the second mixing unit 1-2 to an outlet 6 of the first mixing unit 1-1.

[0191] The connecting part 82 may comprise a second inlet 84 for introducing a third fluid, e.g., a precipitation agent, to the stream exiting the first mixing unit 1-1 in order to be mixed in the second mixing unit 1-2.

[0192] The first mixing unit 1-1 and the second mixing unit 1-2 may be substantially identical or may be different. In particular, the number of branchings and collecting reservoirs may be identical or different.

[0193] In the shown example, both of the first mixing unit 1-1 and the second mixing unit 1-2 each comprise three mixing stages, each including a branching 10, 20, 30, two channels 14-1, 14-2, 24-1, 24-2, 34-1, 34-2 and a collecting reservoir 18, 28, 38.

[0194] **Fig. 9** shows another exemplary mixing unit 1 which may be comprised by a lysis system 90 according to an aspect herein.

[0195] In particular, the mixing unit 1 shown in Fig. 9 comprises substantially the same mixing properties as the mixing system 80 shown in Fig. 8

[0196] Specifically, the mixing unit 1 comprises a first inlet 4 for receiving the inlet stream 5 comprising a first fluid and a second fluid and is configured to mix the same at three mixing stages each including a branching 10, 20, 30, two channels 14-1, 14-2, 24-1, 24-2, 34-1, 34-2 and a collecting reservoir 18, 28, 38.

[0197] However, the mixing unit 1 of Fig. 9 further comprises a second inlet 8 for receiving a third fluid, such as precipitation agent, and introducing said third fluid into the third collecting reservoir 38.

[0198] The mixing unit 1 further comprises a fourth branching 40, quaternary channels 44-1, 44-2, a fourth collecting reservoir 48, a fifth branching 50, quinary channels 54-1, 54-2, a fifth collecting reservoir 58, a sixth branching 60, senary channels 64-1, 64-2 and a sixth collecting reservoir 68 for mixing of the third fluid with the stream of the third collecting reservoir 38 originating from the inlet stream.

[0199] Consequently, the mixing unit 1 shown in Fig. 9 may be considered as integral and/or one-piece version of the mixing system comprising two mixing units 1 shown in Fig. 8.

[0200] A respective mixing unit 1 may be advantageous as it reduces assembly costs and/or improves integrity of the stream. It also efficiently allows influencing the size of flocs, forming during lysis, neutralisation and precipitation by regulating the speed and ratio between fluid streams and gas flow rate. This directly influences the efficiency of the system and enables more efficient filtration between the liquid and sold matter in the following step.

Example 1: In-line lysis of 100 g of *E. coli* cells containing pDNA (4.7 kbp)

CELL RESUSPENSION:

[0201] 100 g of *E. coli* wet cell paste containing plasmid DNA of 4.7 kbp was weighted and diluted with resuspension buffer to the desired resuspension factor (e.g. 20-fold dilution). Then, using a magnetic stirrer, a cell suspension was homogenised (rpm range 200 rpm); to a concentration of 0.05 g/mL.

LYSIS

[0202] Lysis buffer contained sodium hydroxide (0.2 M) and sodium dodecyl sulphate (1%). Feed 1 (resuspended cells) was pumped with a peristaltic pump at a flow rate of 200 mL/min (Fig. 7, 102 and 103); feed 2 (lysis buffer) was pumped with a peristaltic pump at 200 mL/min (Fig. 7, 104 and 105); lysis was performed in a chamber mixer 92, followed by a fixed-volume (1 L) tube reactor (93) at a combined flow rate of 400 mL/min. This achieved a lysis time of 2.5 min.

NEUTRALISATION

[0203] Concentrated K-acetate and was added in a 1:1 ratio with the lysis solution at the T-connector (Fig.7, 96). Feed of 3 M K-acetate, pH 5.5 was pumped with a peristaltic pump at 200 mL/min (Fig.7, 106 and 107) to reach a final concentration of 1 M K-acetate. Simultaneously, an air feed with a flow rate of 0.75 L/min is added through the third inlet (Fig.7, 98). Crude lysate was mixed with this neutralization mixture in a mixing unit (Fig.7, 1).

PRECIPITATION

[0204] Concentrated $CaCl_2$ solution was added to a neutralized lysate using a second inlet of connecting part inlet between 2 mixing units (Fig.7, 1). Feed of 5 M $CaCl_2$ was pumped with a peristaltic pump at 106 mL/min (Fig.7, 108 and 109) to reach a final concentration of 0.75 M $CaCl_2$. Neutralized lysate was mixed with this precipitation agent in a mixing unit (Fig.7, 1). The suspension was collected in reservoir 100 in Fig. 7.

[0205] An aliquot of collected neutralized and precipitated lysate was taken, centrifuged at 13000 rpm for 5 min. The supernatant was collected and diluted 50 times before analysis by HPLC pDNA analytics (using CIMac pDNA column).

Example 2: Batch lysis of 1 g of *E. coli* cells

[0206] Recovery and purity of isolated plasmid was compared to batch lysis of 1 g of *E.* coli cells from the same batch as used for the in-line lysis experiment. HPLC analysis of the product after lysate clarification (Fig. 10) demonstrated comparable plasmid recovery from in-line lysis of 100 g of cells and batch lysis of 1 g of cells. An aliquot of collected neutralized and precipitated lysate was taken, centrifuged at 13000 rpm for 5 min. The supernatant was collected and diluted 50 times before analysis by HPLC pDNA analytics (using CIMac pDNA column). HPLC analysis of the product after lysate clarification demonstrated comparable plasmid recovery from in-line lysis of 100 g of cells (4 mg/g isolated) and batch lysis of 1 g of cells (3.99 mg/g isolated), despite the 100-fold higher scale. Product purity was higher with in-line lysis approach (1.2 times lower genomic DNA content).

[0207] Fig. 10 shows analytical HPLC chromatograms (CIMac pDNA column) comparing product quality and quantity of 4.7 kbp plasmid isolated from *E. coli* using batch lysis (1 g of *E. coli* cell paste; full line) and in-line lysis (100 g *E. coli* cell paste; dashed

List of Reference Numerals

[0208]

1	mixing unit
2	body
3	longitudinal axis of mixing unit
4	first inlet
5	inlet stream
6	outlet
8	second inlet of mixing unit
10	first branching
12-1	first primary branch stream
12-2	second primary branch stream
14-1	first primary channel
14-2	second primary channel
15	entrance section of first/second primary channel
15a	entrance angle
16	exit section of first/second primary channel
16a	exit angle
17	reduced diameter portion of first/second primary channel
18	first collecting reservoir
20	second branching
22-1	first secondary branch stream
22-2	second secondary branch stream
24-1	first secondary channel
24-2	second secondary channel
25	entrance section of first/second secondary channel
26	exit section of first/second secondary channel
27	reduced diameter portion of first/second secondary channel
28	second collecting reservoir
30	third branching
32-1	first tertiary branch stream
32-2	second tertiary branch stream
34-1	first tertiary channel
34-2	second tertiary channel
35	entrance section of first/second tertiary channel

36	exit section of first/second tertiary channel
37	reduced diameter portion of first/second tertiary channel
38	third collecting reservoir
40	fourth branching
44-1	quaternary first channel
44-2	quaternary second channel
48	fourth collecting reservoir
50	fifth branching
54-1	quinary first channel
54-2	quinary second channel
58	fifth collecting reservoir
60	sixth branching
64-1	senary first channel
64-2	senary second channel
68	sixth collecting reservoir
80	mixing system
82	connecting part
84	second inlet of connecting part
90	lysis system
92	mixing chamber
94	incubation tube
96	first joint
98	third inlet
100	collecting vessel
102	first tank
103	first pump
104	second tank
105	second pump
106	third tank
107	third pump
108	fourth tank
109	fourth pump

Claims

1. Mixing unit for mixing two or more fluids, in particular fluids containing shear sensitive biological material, the mixing unit comprising:

a first inlet for receiving an inlet stream, the inlet stream comprising a first fluid and a second fluid;
a channel structure in fluid communication with the inlet, the channel structure comprising:

a first branching for splitting the inlet stream into a first and a second primary branch streams;
a first and a second primary channel, each configured for piping of one of respective primary branch streams,
a first collecting reservoir for recombining the primary branch streams;
wherein the first and second primary channels fluidly connect the first branching with the first collecting reservoir.

2. Mixing unit of claim 1, wherein the channel structure further comprises:

a second branching for splitting the stream in the first collecting reservoir into a first and a second secondary branch streams;
a first and a second secondary channel, each configured for piping of one of respective secondary branch streams;
a second collecting reservoir for recombining the secondary branch streams;
wherein the first and second secondary channels fluidly connect the second branching with the second collecting reservoir,
optionally wherein the primary channels and the secondary channels are arranged in a common plane; and/or wherein the first branching, the first collecting reservoir, the second branching and the second collecting reservoir are positioned on a common axis.

3. Mixing unit of any one of the preceding claims, wherein the mixing unit is configured to:

achieve low shear mixing of the components of the inlet stream; and/or
mix the components of the inlet stream at a low Reynolds number, preferably at a Reynolds number between approximately 6000 and approximately 70000.

4. Mixing unit of any one of the preceding claims, wherein:

an entrance angle between the main flow directions of the branch streams at respective entrance sections of the first and second channels at the associated branchings is substantially between approximately 40° and approximately 120°, preferably between approximately 70° and approximately 90°, and, preferably, is oriented symmetrically with respect to a longitudinal axis of the mixing unit; and/or
an exit angle between the main flow direction of the branch stream at an exit section of the first and second channels at the associated collecting reservoirs is substantially between approximately 40° and approximately 120°, preferably between approximately 70° and approximately 90°, and, preferably, is oriented symmetrically with respect to a longitudinal axis of the mixing unit.

5. Mixing unit of any one of the preceding claims, wherein:

the primary and/or secondary channels comprise one or more sections having a substantially circular and/or oval cross-sectional shape;

and/or
the channels comprise a reduced diameter portion with a reduced cross-section substantially orthogonal to a flow direction of the stream compared to an average cross-section, preferably at a position closer to the associated collecting reservoir than to the associated branching.

6. Mixing unit of any one of the preceding claims, wherein:

wherein the volumes of the first and second primary channels are substantially identical; and/or
wherein the volumes of the first and second secondary channels are substantially identical; and/or
the first and second primary channels are substantially congruent; and/or
the first and second secondary channels are substantially congruent; and/or
the primary channels and the secondary channels are substantially congruent; and/or.
the primary channels are positioned substantially symmetrically with respect to a longitudinal axis of the mixing unit; and/or
the secondary channels are positioned substantially symmetrically with respect to a longitudinal axis of the mixing unit.

7. Mixing unit of any one of the preceding claims, further comprising a second inlet provided at one of the collecting reservoirs for introducing a third fluid into the respective collecting reservoir.

8. Use of the mixing unit of any one of the preceding claims for substantially continuous processing of one or more of: cell lysis, neutralization, clarification, nucleic acid purification and concentration.

9. Mixing system for mixing two or more fluids, the mixing system comprising:

a first mixing unit according to any one of claims 1 to 7; and
a second mixing unit according to any one of claims 1 to 7;
wherein the first mixing unit and the second mixing unit are fluidly connected in series wherein the first inlet of the second mixing unit is fluidly connected to an outlet of the first mixing unit.

10. Mixing system of claim 9, further comprising a connection part for fluidly connecting the outlet of the first mixing unit with the first inlet of the second mixing unit, wherein the connection part comprises a second inlet for receiving a third fluid.

11. Method of mixing two or more fluids, in particular fluids containing shear sensitive biological material and/or their components, the method comprising the steps of:

splitting an inlet stream comprising a first fluid and a second fluid into a plurality of primary branch streams; and
recombining the plurality of primary branch streams in a first collecting reservoir.

12. Method of claim 11, comprising the steps of:

splitting the stream in the first collecting reservoir into a plurality of secondary branch streams; and
recombining the plurality of secondary branch streams in a second collecting reservoir.

13. Method of claim 11 or 12, wherein:

the components of the stream are mixed at low shear; and/or
the components of the stream are mixed at a low Reynolds number, preferably at a Reynolds number between approximately 6000 and approximately 70000; and/or
wherein a time of the stream between a first splitting and a final recombining is between approximately 0.1 seconds and approximately 30 seconds, preferably between approximately 1 seconds and approximately 15 seconds; and/or
wherein a flow rate of the stream is between approximately 50 mL/min and approximately 20 L/min, preferably between approximately 150 mL/min and approximately 2000 mL/min; and/or
wherein an average flow velocity of the stream is between approximately 0.03 m/s to approximately 0.4 m/s.

14. Lysis system for lysing cells containing double stranded DNA, the lysing unit comprising:

a mixing system according to claim 9 or 10 or a mixing unit according to any one of claims 1 to 7 having a second inlet for introducing a precipitation agent to the mixed components of the inlet stream;
a mixing chamber for mixing of the first fluid of the inlet stream comprising a lysis buffer and a fluid comprising resuspended cells;
an incubation tube fluidly connected to an outlet of the mixing chamber for piping of the first fluid of the inlet stream during lysis reaction of the cells; and
a first joint for joining the first fluid and the second fluid of the inlet stream, wherein the first joint is fluidly connected to the first inlet of the first

mixing unit of the mixing system respectively to the mixing unit.

15. Method for lysing cells containing double stranded DNA comprising the steps of:

mixing a fluid stream comprising:

a first fluid comprising resuspended cells and a lysis buffer; and
a second fluid comprising a neutralization buffer in a first mixing unit;

adding a third fluid comprising a precipitation agent to the mixed first fluid and second fluid; and
mixing of the first fluid, the second fluid and the third fluid in the first mixing unit or in a second mixing unit.

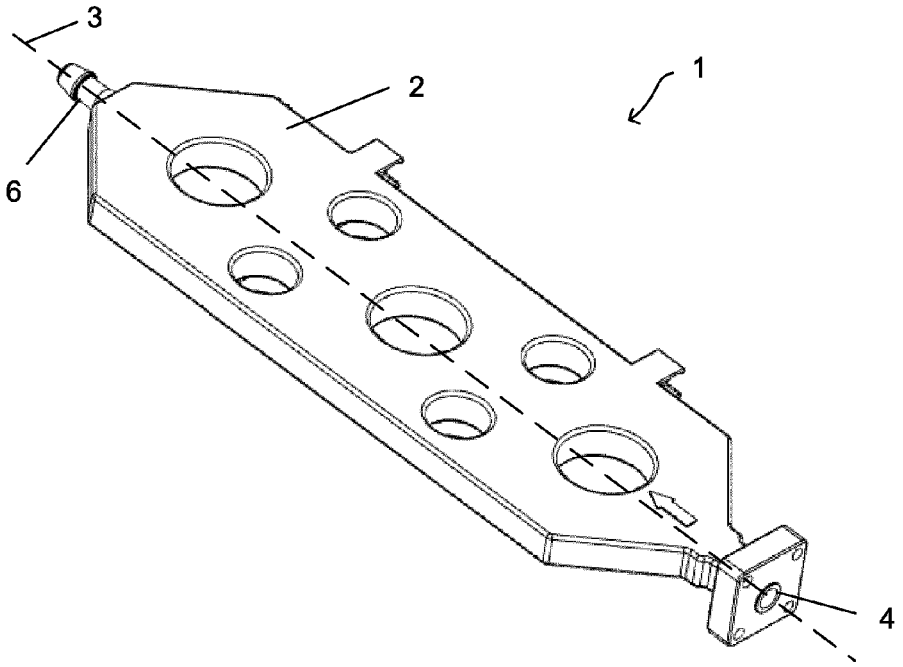


Fig. 1

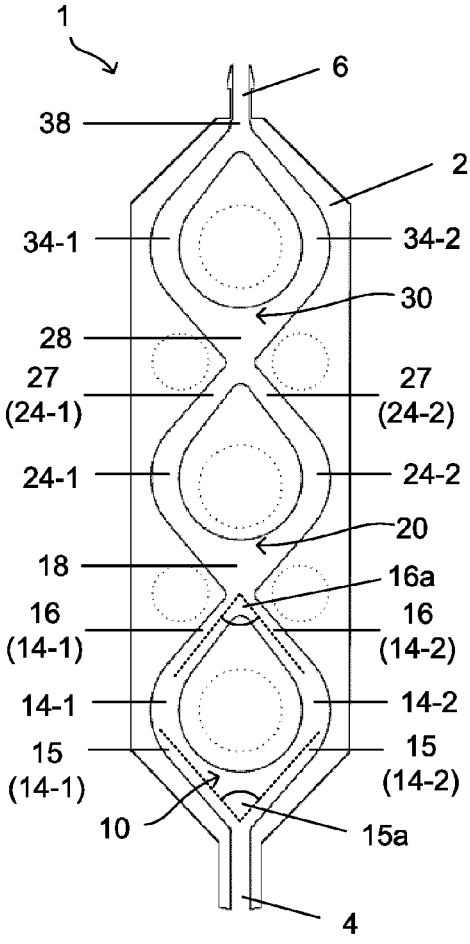


Fig. 2

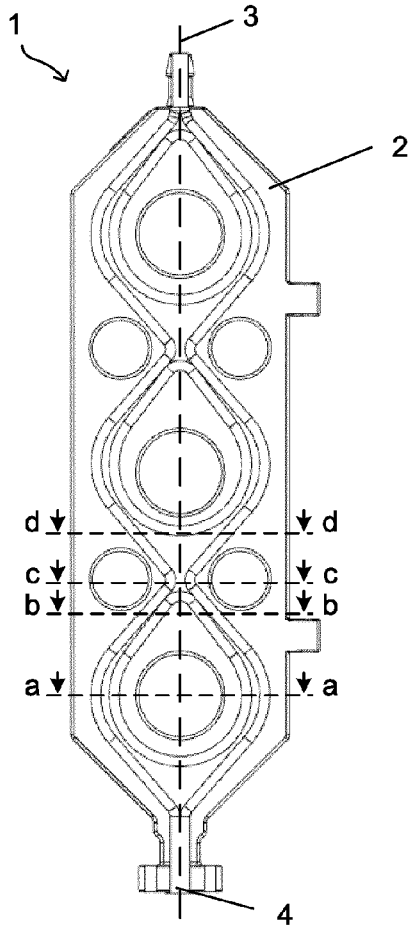


Fig. 3

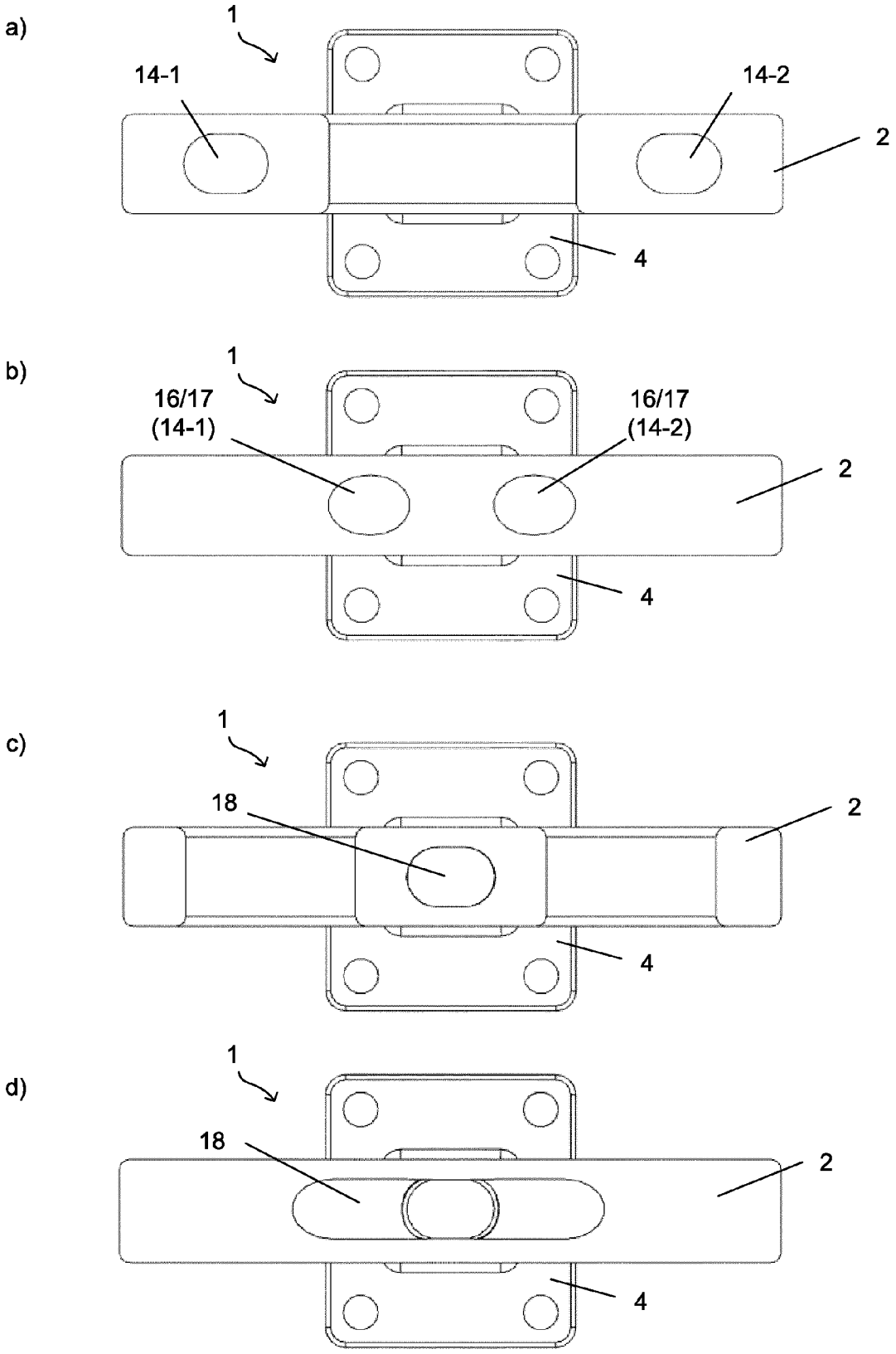
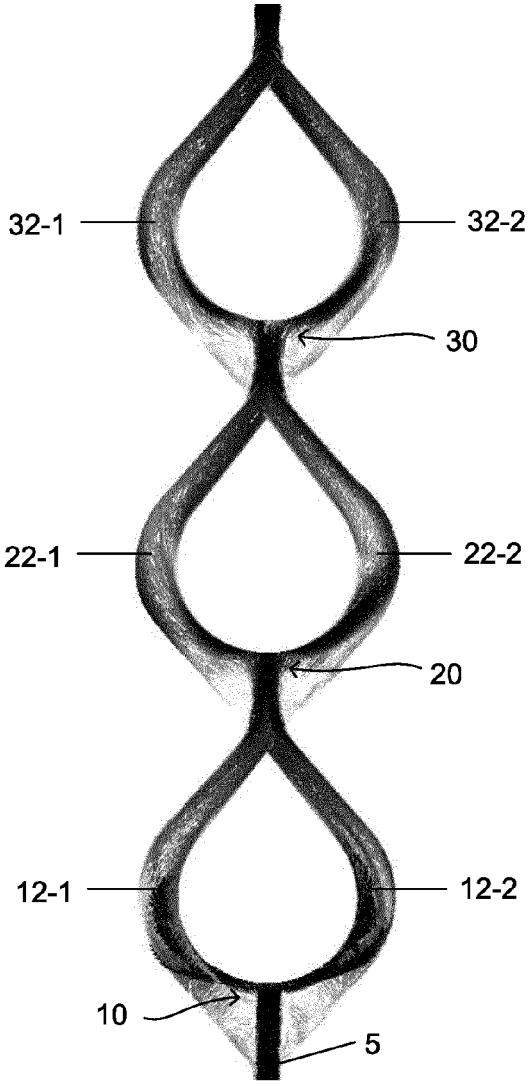


Fig. 4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22-1
22-2
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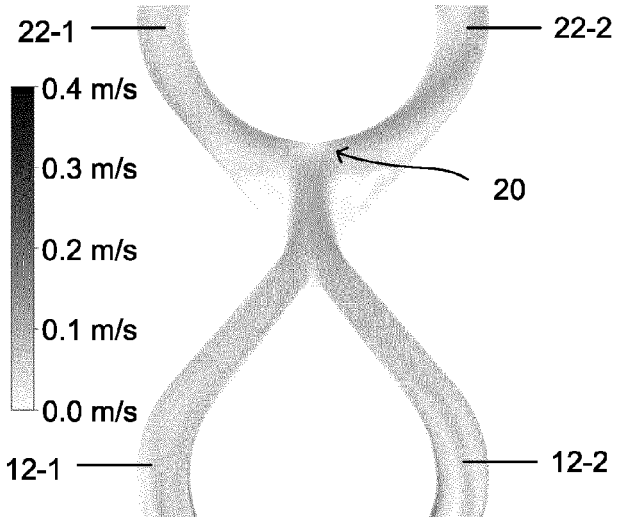
12-1
12-2
10
5

Fig. 5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22-2
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0.3 m/s
0.2 m/s
0.1 m/s
0.0 m/s
20
12-1
12-2

Fig. 6

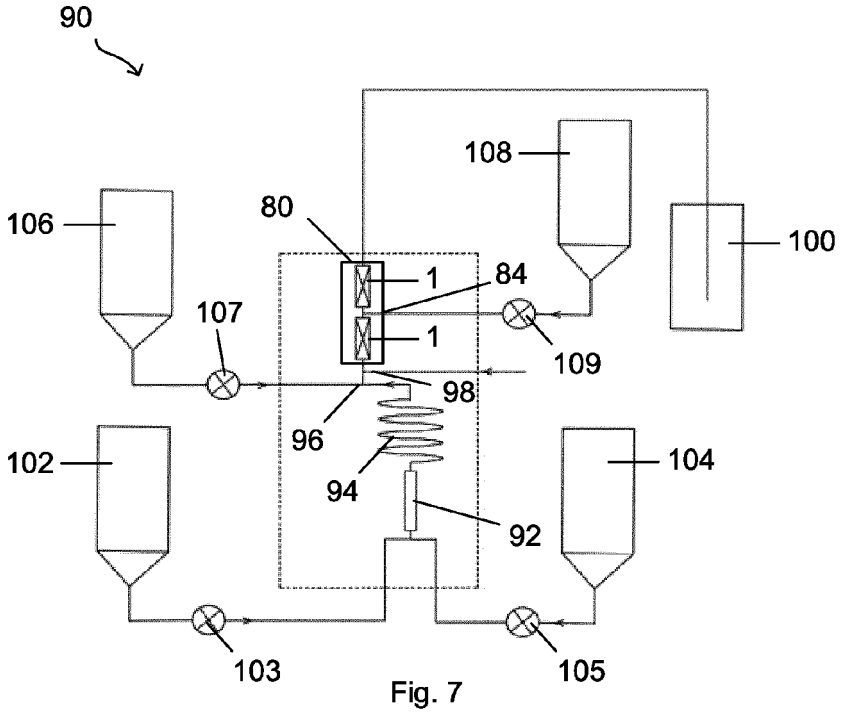


Fig. 7

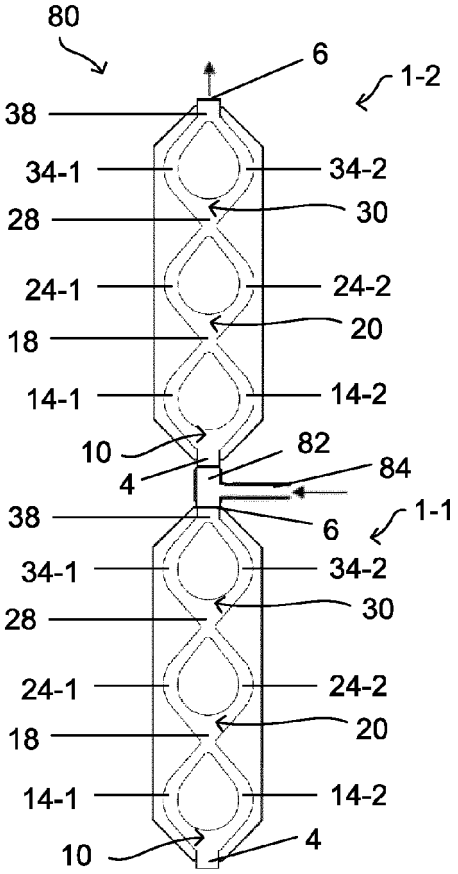


Fig. 8

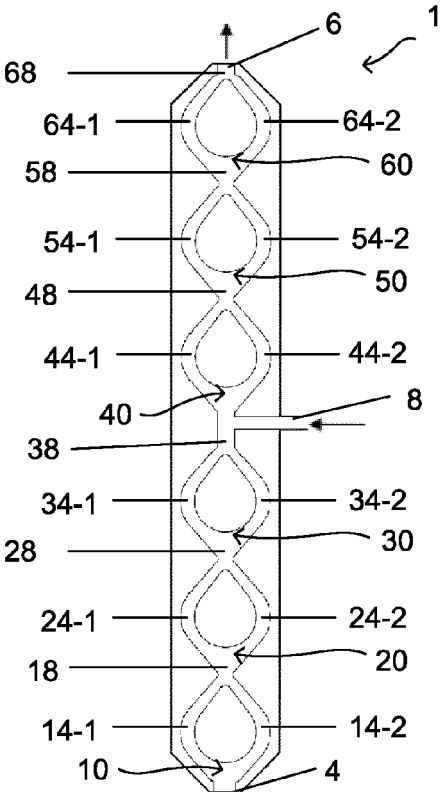
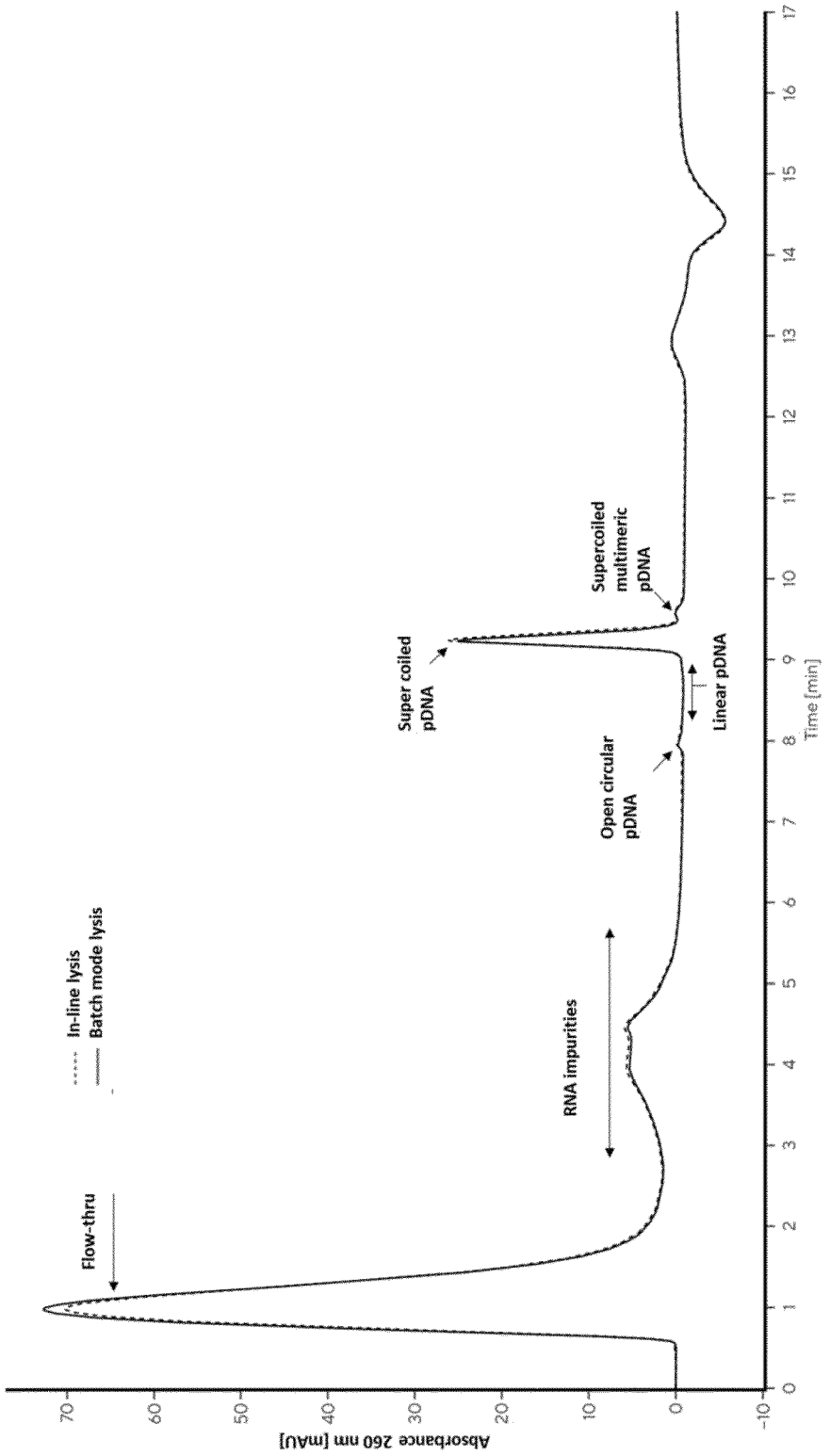


Fig. 9

Fig. 10


In-line lysis
Batch mode lysis
Flow-thru
RNA impurities
Open circular pDNA
Super coiled pDNA
Linear pDNA
Supercoiled multimeric pDNA
Absorbance 260 nm [mAU]
Time [min]

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EUROPEAN SEARCH REPORT

Application Number
EP 23 21 2071

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	US 2006/280029 A1 (GARSTECKI PIOTR [US] ET AL) 14 December 2006 (2006-12-14)	1-3,5,6, 8,9, 11-13	INV. B01F25/432 B01F33/30
A	* paragraph [0002] - paragraph [0003] * * paragraph [0006] - paragraph [0007] * * paragraph [0035] - paragraph [0036] * * paragraph [0049] * * paragraph [0055] - paragraph [0056] * * paragraph [0068] - paragraph [0069] * * figures 1,7d *	4,7,10, 14,15	B01F33/81 B01F35/71
X	WO 99/09042 A2 (CEPHEID [US]) 25 February 1999 (1999-02-25)	1-4,6,8, 9,11-13	
A	* page 1, line 14 - page 2, line 6 * * page 6, line 2 - line 28 * * page 11, line 34 - page 12, line 6 * * page 16, line 1 - line 18 * * page 19, line 27 - page 20, line 6 * * figure 4 *	5,7,10, 14,15	
X	DE 197 46 583 A1 (MERCK PATENT GMBH [DE]) 29 April 1999 (1999-04-29)	1-6,9, 11-13	TECHNICAL FIELDS SEARCHED (IPC)
A	* column 1, line 3 - line 40 * * column 2, line 44 - column 3, line 6 * * figures *	7,8,10, 14,15	B01F
X	US 10 537 869 B1 (YOUNG MU-JEN [TW] ET AL) 21 January 2020 (2020-01-21)	1-14	
A	* figures 3,4 *	15	
X	US 2009/087509 A1 (LINARES MIGUEL [US]) 2 April 2009 (2009-04-02)	1-7,9-14	
A	* figure 4 *	8,15	

-/--

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	27 August 2024	Real Cabrera, Rafael

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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P : intermediate document

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D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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EUROPEAN SEARCH REPORT

Application Number
EP 23 21 2071

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
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A	* page 15, line 27 - page 16, line 5 * * page 23, line 17 - page 24, line 19 * * page 93, line 4 - page 96, line 28 * -----	1-14	
X	US 9 248 422 B2 (CHING JESUS [US]; LEE PHILLIP YOU FAI [US] ET AL.) 2 February 2016 (2016-02-02)	15	
A	* column 1, line 33 - line 42 * * column 26, line 14 - line 33 * * column 27, line 38 - line 59 * * column 31, line 1 - line 8 * * column 73, line 34 - line 60 * -----	1-14	
			TECHNICAL FIELDS SEARCHED (IPC)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	27 August 2024	Real Cabrera, Rafael

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L : document cited for other reasons

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Application Number
EP 23 21 2071

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing claims for which payment was due.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

☐ The present supplementary European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims (Rule 164 (1) EPC).

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LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 23 21 2071

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-4, 6, 8, 9, 11-13(completely); 5(partially)

   Mixing unit having branched channel structure, wherein the entrance angle of the branch streams at entrance and/or exit sections is between 40° and 120°.

   ---

2. claim: 5(partially)

   Mixing unit having branched channel structure, wherein the channels comprise a reduced diameter portion with a reduced cross section.

   ---

3. claims: 7, 10, 14

   Mixing unit having branched channel structure, wherein the mixing unit comprises a second inlet provided at one of a collecting reservoir for introducing a third fluid.

   ---

4. claim: 15

   Method for lysing cells containing double stranded DNA.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 23 21 2071

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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27-08-2024

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