CLAIMS

What is claimed is:

1. A system for continuous in-line lysing of cells containing plasmid DNA, the system comprising:

a single-use flow path comprising:

a first container comprising a first fluid comprising cells containing plasmid nucleic acids (**1**), the first container being fluidly connected via a first pump (**P1**) to a first single-use fluidic pathway (**1a**);

a second container (**2**) comprising a second fluid that is an alkaline lysis buffer fluidly connected via a second pump (**P2**) to the second single-use fluidic pathway (**2a**);

the first and second fluids being combined, directly or using a mixer, into a third single-use fluidic pathway (**3a**);

the third fluidic pathway (**3a**) being fluidly connected to at least one third container (**3**) comprising single-use tubing within which the cells are at least partially lysed to release the plasmid nucleic acids and, if more than one third container (**3b**) is present, each third container being fluidly connected to one another in series by one or more additional single-use fluidic pathways (**3b**) and comprising an exit fluidly connected to a fourth single-use fluidic pathway (**3c**);

a fourth container (**4**) comprising neutralization buffer fluidly connected via a third pump (**P3**) to the single-use flow path through a fifth single-use fluidic pathway (**4a**); and,

the fourth (**3c**) and fifth (**4a**) single-use fluidic pathways being combined, directly or using a mixer, into a sixth single-use fluidic pathway (**5**) containing final product comprising released plasmids and cell debris;

wherein:

the fluids in the first (**1a**) and second single use fluidic pathways (**2a**) are combined into the third fluidic pathway (**3a**) that is fluidly connected to the at least one third container;

the fluid in the third container (**3/ 3b**) is maintained therein for a sufficient amount of time to lyse cells and release plasmids from the cells while minimizing

degradation of the plasmids, and exits the retention tubing section through the fourth fluidic pathway (**3c**);

the fluid of the fourth fluidic pathway is combined, directly or using a mixer, with neutralization buffer from the fourth container (**4**) in the fifth fluidic pathway (4a) and exits the single-use flow path through a seventh fluidic pathway (**6**); and

the single-use tubing of the third container (**3**) comprising single-use tubing within which the cells are at least partially lysed to release the plasmid nucleic acids, wherein the single-use tubing comprises at least one mixing baffle therein.

2. The system of claim 1 wherein the tubing comprises a mixing baffle provided by at least one first indentation positioned at approximately the midpoint of the fluidic conduit, optionally further comprising at least one second indentation being positioned between the first and the fluid exit located opposite the fluid entry of the retention module.

3. The system of claim 2 wherein the third container (**3**) comprises one fluidic entrance and one fluidic exit.

4. The system of claim 2 wherein the third container (**3**) comprises one fluidic entrance and at least two fluidic exits.

5. The system of any preceding claim wherein the flow of cells, lysis buffer, and/or neutralization buffer is controlled by a process controller such as a Programmable Logic Controller (PLC) or Distributed Control System (DCS), with a Human-Machine Interface (HMI) used for inputting process recipe parameters, monitoring the process, and collecting process data.

6. The system of claim 5 wherein the at least one inline analytical instrument measures pH and/or conductivity of at least one fluid, buffer and/or stream.

7. The system of any preceding claim wherein the flow of cells, lysis buffer, and/or neutralization buffer is controlled via feedback from inline analytical instruments such as but not limited to pH and conductivity.

8. The system of any preceding claim wherein the pH of the alkaline lysis buffer is less than 13 (pH<13), optionally wherein the alkaline lysis buffer is 0.1-0.2 N NaOH.

9. The system of any preceding claim wherein the sufficient amount of time is two to ten minutes.

10. The system of any preceding claim wherein the retention tubing section comprises pre-packaged disposable tubing within a rigid container.

11. The system of any preceding claim wherein the retention tubing section comprises multiple subsections fluidly connected to one another, each subsection comprising pre-packaged disposable tubing within a rigid container, the length and/or type of disposable tubing of each subsection being the same or different from the length and/or type of disposable tubing of at least one of the other subsections.

12. The system of any preceding claim wherein the fourth fluidic pathway comprises a static mixer.

13. The system of any preceding claim wherein the exit port is fluidly connected to a fifth fluidic pathway that is fluidly connected a container or another piece of equipment.

14. A method for isolating plasmid DNA from a cell culture, the method comprising processing cells comprising plasmid DNA through a system and/or reactor module of any preceding claim.