CLAIMS

What is claimed is:

1. A microfluidic apparatus for forming a therapeutic polynucleotide, the apparatus comprising:

    a seating mount for removably holding a microfluidic path device;

    a plurality of pressure lines;

    a plurality of fluid vials, wherein each fluid vial either comprises a fluidic line or is configured to couple with the fluidic line,

    wherein each fluidic line and at least a subset of the pressure lines are configured to be biased against the microfluidic path device held in the seating mount to form a closed fluid path; and

    a controller configured to control the application of pressure through the pressure lines to drive fluidic movement in the microfluidic path device when the microfluidic path device is held in the seating mount, wherein the controller is configured to direct the synthesis of a synthetic template, direct an *in vitro* transcription (IVT) reaction using the template to form a therapeutic polynucleotide, and direct purification of the therapeutic polynucleotide in one or more microfluidic path devices held in the seating mount.

2. A microfluidic apparatus for forming a therapeutic polynucleotide, the apparatus comprising:

    a seating mount for removably holding a microfluidic path device;

    a plurality of pressure lines;

    a plurality of fluid vials, wherein each fluid vial either comprises a fluidic line or is configured to couple with the fluidic line,

    wherein each fluidic line and at least a subset of the pressure lines are configured to be biased against the microfluidic path device held in the seating mount to form a closed fluid path; and

    a controller configured to control the application of pressure through the pressure lines to drive fluidic movement in the microfluidic path device when the microfluidic path device is held in the seating mount, wherein the controller is

configured to determine the contents of the fluid vials, transfer sub-microliter amounts of material from the fluid vials to one or more reactors in the microfluidic path device held in the seating mount, direct the synthesis of a synthetic template, direct an *in vitro* transcription (IVT) reaction using the template to form a therapeutic polynucleotide, and direct purification of the therapeutic polynucleotide in one or more microfluidic path devices held in the seating mount.

3. The apparatus of claims 1 or 2, further comprising a reagent storage frame holding the plurality of fluid vials.

4. The apparatus of any of claims 1-3, further comprising a plurality of optical sensors arranged to monitor fluid levels within the plurality of fluid vials and to monitor fluidic movement in the microfluidic path device when the microfluidic path device is held in the seating mount.

5. The apparatus of any of claims 1-4, further comprising a fluid interface assembly configured to hold the fluidic lines.

6. The apparatus of any of claims 1-5, further comprising a fluid interface assembly release control configured to release the fluid interface assembly from the apparatus.

7. The apparatus of any of claims 1-6, further comprising a reagent storage frame release control configured to release the reagent storage frame from the apparatus.

8. The apparatus of any of claims 1-7, further comprising a thermal control configured to modulate the temperature of at least one region of the microfluidic path device when the microfluidic path device is held in the seating mount.

9. The apparatus of any of claims 1-8, further comprising a magnetic field applicator configured to apply a magnetic field to at least one region of the microfluidic path device when the microfluidic path device is seated in the seating mount.

10. The apparatus of claim 9, wherein the magnetic field applicator comprises a control arm mounted to the reagent storage frame.

11. The apparatus of any of claims 1-10, wherein the controller is configured to detect an identifying code on the fluid vial.

12. The apparatus of any of claims 1-11, wherein the controller is configured to determine a level of a reagent held by one or more of the plurality of fluid vials.

13. The apparatus of any of claims 1-12, further comprising an optical sensor drive configured to move one or more of a plurality of optical sensors around the seating mount or around the plurality of vials.

14. The apparatus of any of claims 1-13, further comprising one or more alignment pins configured to align the microfluidic path device in the seating mount.

15. The apparatus of any of claims 1-14, further comprising an enclosure having a cover, configured to maintain the sterility of the apparatus during operation of the apparatus.

16. The apparatus of any of claims 1-15, further comprising the microfluidic path device.

17. The apparatus of any of claims 1-16, further comprising a fluid interface assembly having a central opening through which the microfluidic path device may be imaged by one or more of a plurality of optical sensors, further wherein a sealing end of each of the fluidic lines and pressure lines are arranged around a periphery of the central opening.

18. The apparatus of any of claims 1-17, further comprising a signal detector configured to detect a signal from within the microfluidic path device.

19. The apparatus of claim 18, wherein the signal is one or more of: a visible, fluorescent, UV absorbance or IR absorbance signal.

20. The apparatus of claim 18, wherein the signal detector is configured to measure a nanoparticle size distribution.

21. A microfluidic apparatus for forming a therapeutic polynucleotide, the apparatus comprising:

a seating mount for removably holding a microfluidic path device;

a plurality of pressure lines;

a plurality of fluid vials that are each pressurized by one or more pressure lines from the plurality of pressure lines, further wherein each fluid vial either comprises a fluidic line or is configured to couple with the fluidic line,

wherein each fluidic line and at least a subset of the pressure lines are configured to be independently biased against the microfluidic path device seated in the seating mount to form a sealed closed fluid path; and

a controller configured to control the application of pressure through the pressure lines to drive fluidic movement in the microfluidic path device when the microfluidic path device is seated in the seating mount and to apply pressure to one or more of the pressure lines to open or close valves in the microfluidic path device during operation.

22. A microfluidic apparatus for forming a therapeutic polynucleotide, the apparatus comprising:

a seating mount for a microfluidic path device;

a plurality of pressure lines;

a fluid interface assembly comprising a plurality of fluidic lines;

a plurality of fluid vials configured to be pressurized;

a reagent storage frame comprising a plurality of holders each configured to hold a fluid vial of the plurality of fluid vials, wherein each fluid vial either comprises a fluidic line of the plurality of fluidic lines, or is configured to couple with a fluidic line of the plurality of fluidic lines, further wherein each fluidic line and at least some of the pressure lines are configured to be separately biased against the microfluidic path device seated in the seating mount with a bias force; and

a controller configured to control the application of pressure through the pressure lines to drive fluidic movement in the microfluidic path device when the microfluidic path device is seated in the seating mount.

23. A microfluidic apparatus for forming a therapeutic polynucleotide, the apparatus comprising:

a seating mount for removably holding a microfluidic path device;

a plurality of pressure lines wherein at least a subset of the pressure lines are configured to be independently biased against a pressure input on the microfluidic path device seated in the seating mount;

a plurality of fluid vials configured to be pressurized, further wherein each fluid vial either comprises a fluidic output configured to seal against an input on the microfluidic path device or is configured to couple with a fluidic line that is configured to be independently biased against the microfluidic path device to form a sealed closed fluid path;

a first optical detector configured to monitor fluid within the fluid vials;

a second optical detector configured to monitor fluid within the microfluidic path device seated in the seating mount;

a controller configured to receive input from the first optical detector and the second optical detector and to control the application of pressure through the pressure lines to apply pressure from the plurality of pressure lines to open and/or close valves and to drive fluidic movement in the microfluidic path device based at least in part on the received input.

24. A microfluidic path device comprising:

an elastic layer sandwiched between a first surface and a second surface;

a plurality of chambers formed between the first surface and the second surface, wherein a portion of the elastic layer divides each chamber into a fluid-contacting side in the second surface and a pressure-receiving side in the first surface;

a plurality of fluid channels each extending from a fluid port, the elastic membrane and through the first surface, and into the second surface to fluidly connect with the fluid-contacting sides of the plurality of chambers; and

a plurality of pressure channels each extending from a one or more pressure port, through the first surface and the elastic layer, into the second surface and back through the elastic layer into the first surface, wherein each pressure channel of the plurality of pressure channels fluidly connects with one or more pressure-receiving sides of the plurality of chambers,

further wherein the volumes of the fluid-contacting sides of each chamber may be adjusted by applying pressure from the one or more of the pressure ports.

25. A microfluidic path device for forming a therapeutic polynucleotide, the device comprising:

an elastic layer sandwiched between a first plate region having a first surface and a second plate region having a second surface;

a plurality of chambers each having a fixed volume and formed between the first surface and the second surface, wherein a portion of the elastic layer divides each chamber into a fluid-contacting side in the second surface and a pressure-receiving side in the first surface;

a plurality of fluid channels each extending from a fluid port through the first plate region and into the second plate region to fluidly connect with the fluid-contacting side of one or more of the plurality of chambers; and

a plurality of pressure channels each extending from one or more pressure ports, through the first plate region and elastic layer, into the second plate region, and back through the elastic layer and into the first plate region, wherein each pressure channel of the plurality of pressure channels extends within the first plate region and fluidly connects with one or more pressure-receiving sides of one or more of the plurality of chambers,

wherein the plurality fluid-contacting sides of the plurality of chambers are interconnected, further wherein the fluid-contacting side of each chamber is concave so that the elastic layer seats flush and without gaps to the fluid-contacting side in the second surface when a positive pressure in the pressure-receiving side drives the elastic layer against the fluid-contacting side.

26. A microfluidic path device for forming a therapeutic polynucleotides in a closed path, the microfluidic path device comprising:

an elastic layer sandwiched between a first plate region having a first surface and a second plate region having a second surface;

a plurality of chambers each having a fixed volume and formed between the first surface and the second surface, wherein a portion of the elastic layer divides each chamber into a fluid-contacting side in the second surface and a pressure-receiving side in the first surface;

a plurality of fluid channels each extending from a fluid port through the first plate region and into the second plate region to fluidly connect with the fluid-contacting side of one or more of the plurality of chambers; and

a plurality of pressure channels each extending from one or more pressure ports, through the first plate region and elastic layer, into the second plate region, and back through the elastic layer and into the first plate region, wherein each pressure channel of the plurality of pressure channels extends within the first plate region and fluidly connects with one or more pressure-receiving sides of one or more of the plurality of chambers,

wherein each fluid port comprises a port channel that extends extending transversely through the first plate region, and opens onto an opening through the elastic layer having an opening diameter that is smaller than a diameter of the port channel, further wherein a diameter of the fluid channel second plate region is smaller than the diameter of the port channel.

27. A microfluidic path device for processing therapeutic polynucleotides in a closed path, the microfluidic path device comprising:

an elastic layer sandwiched between a first plate region having a first surface and a second plate region having a second surface;

a plurality of chambers each having a fixed volume and formed between the first surface and the second surface, wherein a portion of the elastic layer divides each chamber into a fluid-contacting side in the second surface and a pressure-receiving side in the first surface;

a plurality of fluid channels each extending from a fluid port through the first plate region and into the second plate region to fluidly connect with the fluid-contacting side of one or more of the plurality of chambers; and

a plurality of pressure channels each extending from one or more pressure ports, through the first plate region and elastic layer, into the second plate region, and back through the elastic layer and into the first plate region, wherein each pressure channel of the plurality of pressure channels extends within the first plate region and fluidly connects with one or more pressure-receiving sides of one or more of the plurality of chambers,

at least one vacuum cap between at least some of the plurality chambers, wherein the at least one vacuum caps comprises a bubble-removing chamber formed between the first surface and the second surface, wherein the elastic layer divides the bubble-removing chamber into a fluid-contacting side of the bubble-removing chamber in the second surface and a vacuum receiving side in the first surface, further wherein the fluid-contacting side of the bubble-removing chamber is in fluid communication with at least two of the fluid-contacting sides of the plurality of chambers and wherein at least the portion of the elastic layer between the fluid-contacting side of the bubble-removing chamber and the vacuum receiving side is gas permeable.

28. A method of processing a fluid in a microfluidic path device to form a therapeutic polynucleotide, the method comprising:

sealingly and independently coupling a distal end of each of a plurality of fluid lines and a plurality of pressure lines to plurality of fluid ports or pressure ports on a surface of a microfluidic path device, wherein each distal end is biased to be driven against an elastic layer between a first surface and a second surface,

wherein the microfluidic path device comprises a plurality of chambers each divided into a fluid-contacting side formed in the second surface and a pressure-receiving side formed in the first surface; and

driving fluid through the fluid-contacting sides of the plurality of chambers by the application of positive and negative pressure within the pressure-receiving sides of the chambers to change the sizes of the plurality of fluid-contacting sides.

29. A method of processing a fluid in a microfluidic path device, the method comprising:

sealingly and independently coupling a distal end of each of a plurality of fluid lines and a plurality of pressure lines to plurality of fluid ports or pressure ports on a surface of a microfluidic path device, wherein each distal end is biased to be driven against an elastic layer between a first plate and a second plate,

wherein the microfluidic path device comprises a plurality of chambers each divided into a fluid-contacting side formed in the second plate and a pressure-receiving

side formed in the first plate, wherein the fluid-contacting sides are interconnected; and

driving fluid through the interconnected fluid-contacting sides and operating a valve to meter the movement of fluid between the fluid-contacting sides of the plurality of chambers by the application of positive and negative pressure within the pressure-receiving sides of the chambers to change the sizes of the plurality of fluid-contacting sides.