

CLAIMS

What is claimed is:

1. A method of manufacturing a therapeutic polynucleotide using a system comprising a plurality of fluid depots configured to be secured in sealed fluid communication with one or more microfluidic path devices, the method comprising: transporting reagents between one or more fluid depots of the plurality of fluid depots and a plurality of reactors on the one or more microfluidic path devices in a sealed and closed fluidic path that is protected from atmospheric contact to perform the steps of: forming a synthetic template, performing *in vitro* transcription from the template to produce a therapeutic polynucleotide, and purifying the therapeutic polynucleotide.
2. The method of claim 1, wherein purifying the therapeutic polynucleotide comprises two-dimensional (2D) purification of the therapeutic polynucleotide within one or more of the plurality of reactors.
3. The method of claim 1, further comprising formulating the therapeutic polynucleotide with a delivery vehicle in one or more reactors on the one or more microfluidic path devices to form a therapeutic polynucleotide composition.
4. The method of claim 3, further comprising concentrating the therapeutic polynucleotide composition in the one or more microfluidic path devices.
5. The method of claim 3, wherein the delivery vehicle comprises an amphipathic nanoparticle.
6. The method of claim 5, wherein the amphipathic nanoparticle comprises an amino-lipidated peptoid.
7. The method of claim 1, wherein purifying the therapeutic polynucleotide comprises removing double-stranded mRNA using a cellulose material within the one or more reactors.
8. The method of claim 1, wherein the system automatically and continuously performs the steps of forming the synthetic template, performing *in vitro* transcription from the template, and purifying the therapeutic polynucleotide with optical feedback from one or more sensors of system.
9. The method of claim 1, wherein the therapeutic polynucleotide is an mRNA.

10. The method of claim 1, wherein the therapeutic polynucleotide is an mRNA, a circular RNA or a self-replicating RNA.
11. The method of claim 1, wherein the system transports the reagents between the one or more fluid depots and the plurality of reactors by fluid power.
- 5 12. The method of claim 11, wherein the system pneumatically transports the reagents between the one or more fluid depots and the plurality of reactors.
13. The method of claim 1, wherein the system transports the reagents between the one or more fluid depots and the plurality of reactors by deflecting one or more elastic layers within the one or more microfluidic path devices.
- 10 14. The method of claim 1, wherein the method is performed at a site of care.
15. The method of claim 1, wherein the system automatically and continuously performs the steps of forming a synthetic template, performing *in vitro* transcription from the template to produce a therapeutic polynucleotide, and purifying the therapeutic polynucleotide in less than 3 days.
- 15 16. The method of claim 1, further comprising sealing the fluid depots to the one or more microfluidic path devices and pressurizing the fluid depots before transporting the reagents between the fluid depots and the plurality of reactors on the one or more microfluidic path devices.
17. The method of claim 1, further comprising recording movement of fluid within the one or
20 more microfluidic path devices during the performance of the steps in a file associated with the therapeutic polynucleotide that is manufactured.
18. A therapeutic polynucleotide made using the method of claim 1.
19. A method of manufacturing a therapeutic mRNA using a system comprising a plurality of fluid depots configured to be secured in sealed fluid communication with one or more
25 microfluidic path plate devices, wherein the one or more microfluidic path plate devices comprise a plurality of reactors, the method comprising:
- delivering a template precursor material from one or more fluid depots to a first one or more reactor regions of the plurality of reactors and processing the template precursor material to prepare a template from the template precursor material;

- transferring the template to a second one or more reactor regions of the plurality of reactors and processing the template by *in vitro* transcription to form a therapeutic mRNA; and
- transferring the therapeutic mRNA to a third one or more reactor regions of the plurality of reactors and purifying the therapeutic mRNA by two-dimensional (2D) purification within the third one or more reactor regions;
- wherein all of the method steps are performed without exposing the template and therapeutic mRNA to atmospheric contact.
20. A method of manufacturing a therapeutic mRNA using a system comprising a plurality of fluid depots in sealed fluid communication with one or more microfluidic path plate devices, wherein the one or more microfluidic path plate devices comprise a plurality of reactors, the method comprising:
- delivering, using fluid power, a template precursor material from one or more fluid depots to a first one or more reactor regions of the plurality of reactors and processing the template precursor material to prepare a template from the template precursor material;
- transferring, using fluid power, the template to a second one or more reactor regions of the plurality of reactors and processing the template by *in vitro transcription* to form a therapeutic mRNA;
- transferring, using fluid power, the therapeutic mRNA to a third one or more reactor regions of the plurality of reactors and purifying the therapeutic mRNA by two-dimensional (2D) purification within the third one or more reactor regions;
- transferring, using fluid power, the therapeutic mRNA to a fourth one or more reactor regions of the plurality of reactors and encapsulating the therapeutic mRNA with a delivery vehicle to form a therapeutic mRNA composition; and
- concentrating, using fluid power, the therapeutic mRNA composition in a fifth one or more fluid depots,
- wherein all of the method steps are performed without exposing the template and the therapeutic mRNA to atmospheric contact.
21. A non-transitory computer readable medium embodying instructions for manufacturing a therapeutic polynucleotide, that when executed by a controller of a system comprising a plurality of fluid depots configured to be secured in sealed fluid communication with one or more microfluidic path devices, cause the controller to perform the method of:

- pressurize the plurality of fluid depots in fluid communication with one or more microfluidic path devices;
- transporting reagents between one or more fluid depots of the plurality of fluid depots and a plurality of reactors on the one or more microfluidic path devices in a sealed and
- 5 closed fluidic path that is protected from atmospheric contact to perform the steps of:
- forming a synthetic template,
- performing *in vitro* transcription from the template to produce a therapeutic polynucleotide, and
- purifying the therapeutic polynucleotide.
- 10 22. The computer-readable medium of claim 21, wherein the instructions further cause the controller to automatically and continuously perform the steps of forming the synthetic template, performing *in vitro* transcription from the template, and purifying the therapeutic polynucleotide based on optical feedback from one or more optical sensors of the system.
- 15 23. The computer-readable medium of claim 21, wherein the instructions further cause the controller to control purifying of the therapeutic polynucleotide by two-dimensional (2D) purification within one or more of the plurality of reactors.
- 20 24. The computer-readable medium of claim 21, wherein the instructions further cause the controller to formulate the therapeutic polynucleotide with a delivery vehicle in one or more reactors on the one or more microfluidic path devices to form a therapeutic polynucleotide composition.
- 25 25. The computer-readable medium of claim 21, wherein the instructions further cause the controller to concentrate the therapeutic polynucleotide composition in the one or more microfluidic path devices.
26. The computer-readable medium of claim 21, wherein the instructions further cause the controller to transport the reagents between the one or more fluid depots and the plurality of reactors by fluid power.
27. The computer-readable medium of claim 21, wherein the instructions further cause the controller to transport the reagents between the one or more fluid depots and the plurality of reactors by deflecting one or more elastic layers within the one or more microfluidic path
- 30 devices.

28. The computer-readable medium of claim 21, wherein the instructions further cause the controller to automatically and continuously perform the steps of forming a synthetic template, performing *in vitro* transcription from the template to produce a therapeutic polynucleotide, and purifying the therapeutic polynucleotide in less than 5 days.
- 5 29. The computer-readable medium of claim 21, wherein the instructions further cause the controller to record movement of fluid within the one or more microfluidic path devices during the performance of the steps in a file associated with the therapeutic polynucleotide that is manufactured.
- 10 30. An automated method of making a synthetic double-stranded DNA template for an mRNA synthesis using a closed-path system comprising a plurality of fluid depots configured to be secured in sealed fluid communication with one or more microfluidic path devices, the method comprising:
- 15 transporting reagents between one or more fluid depots of the plurality of fluid depots and a plurality of reactors on the one or more microfluidic path devices in a closed fluidic path that is protected from atmospheric contact to combine the reagents; and
- forming the synthetic double-stranded DNA template for *in vitro* transcription of a therapeutic mRNA.
31. The method of claim 30, wherein the synthetic double-stranded DNA template is free of bacterial DNA and free of endotoxin.
- 20 32. The method of claim 30, further comprising receiving, in a controller for the closed-path system, optical sensor data from one or more sensors of the closed-path system, wherein the controller controls the operation of the closed-path system based on the optical sensor data.
33. The method of claim 30, further comprising pressurizing the fluid depots.
- 25 34. The method of claim 30, wherein transporting reagents comprises transporting a synthetic gene of interest and a synthetic *in vitro* transcription facilitator cassette from one or more fluid depots of the plurality of fluid depots to a first one or more reactors in the microfluidic path device, joining the synthetic gene of interest with the synthetic *in vitro* transcription facilitator cassette to create a synthetic product, removing unreacted material away from the synthetic product, and amplifying the synthetic product to generate the synthetic double-
- 30 stranded DNA template.

35. The method of claim 30, wherein the one or more microfluidic path devices comprises a microfluidic path plate device seated in the closed-path system.
36. An automated method of making a synthetic double-stranded DNA template for *in vitro* transcription using a closed-path system comprising a plurality of fluid depots in sealed fluid communication with a microfluidic path device, the method comprising:
- 5 transporting reagents, including a synthetic gene of interest and a synthetic *in vitro* transcription facilitator cassette, from one or more fluid depots of the plurality of fluid depots to a first one or more reactors in the microfluidic path device in a closed fluidic path that is protected from atmospheric contact;
- 10 joining the synthetic gene of interest with the synthetic *in vitro* transcription facilitator cassette to create a synthetic product;
- transporting the synthetic product in the microfluidic path device to remove unreacted synthetic gene of interest and unreacted synthetic *in vitro* transcription facilitator cassette away from the synthetic product; and
- 15 transporting the synthetic product in the microfluidic path device and amplifying the synthetic product to generate the double-stranded DNA template.
37. The method of claim 36, wherein amplifying the synthetic product generating greater than 1 mM of an amplified DNA template.
38. The method of claim 36, wherein the synthetic double-stranded DNA template is free of bacterial DNA and free of endotoxin.
- 20 39. The method of claim 36, further comprising receiving, in a controller for the closed-path system, optical sensor data from one or more sensors of the closed-path system, wherein the controller controls the operation of the closed-path system based on the optical sensor data.
40. The method of claim 36, further comprising pressurizing the fluid depots.
- 25 41. The method of claim 36, wherein the transporting steps each comprise using one or more fluid power circuits to move material between the plurality of fluid depots and the microfluidic path device or within the microfluidic path device.
42. The method of claim 36, further comprising transferring the amplified DNA template to one or more digestion reactors of the microfluidic path device and enzymatically modifying the amplified synthetic product to generate the double-stranded DNA template.
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43. The method of claim 36, wherein joining the synthetic gene of interest with the synthetic *in vitro* transcription facilitator cassette to create a synthetic product comprises creating a synthetic linear or circular ligated product.
44. The method of claim 36, wherein amplifying the synthetic product comprises generating a linear, branched or circular amplified DNA product, further comprising linearizing the amplified DNA product to generate the double-stranded DNA template.
45. The method of claim 36, wherein joining comprises ligating with a DNA ligase.
46. The method of claim 36, wherein jointing comprises annealing or primer extension.
47. The method of claim 36, wherein amplifying comprises multiple displacement amplification (MDA).
48. The method of claim 36, wherein amplifying comprises polymerase chain reaction (PCR) amplification.
49. The method of claim 36, wherein the synthetic *in vitro* transcription facilitator cassette comprises a double-stranded DNA template comprising a promoter; a 5' UTR; a cleavable linker; a 3' UTR; and a portion encoding a polyA region comprising at least 200 adenine residues or 200 thymidine residues in a row.
50. The method of claims 36 wherein the double-stranded DNA template includes a polyA region is at least 300 bps long at the 3' end of the synthetic gene of interest.
51. The method of claim 36, wherein the *in vitro* transcription facilitator cassette is less than 1 kb in length.
52. The method of claim 36, wherein the synthetic *in vitro* transcription facilitator cassette does not encode an antibiotic resistance gene.
53. The method of claim 36, wherein the *in vitro* transcription facilitator cassette does not have an origin of replication (ORI).
54. An automated method of making a synthetic double-stranded DNA template for *in vitro* transcription using a closed-path system comprising a plurality of fluid depots in sealed fluid communication with a microfluidic path device, the method comprising:
transporting reagents, including a synthetic gene of interest and a synthetic *in vitro* transcription (IVT) facilitator cassette, from one or more fluid depots of the plurality of

- fluid depots to a first one or more reactors in the microfluidic path device in a closed fluidic path that is protected from atmospheric contact;
joining the synthetic gene of interest with the synthetic IVT facilitator cassette in the first one or more reactors to create a synthetic product;
5 transporting the synthetic product to a second one or more reactors in the microfluidic path device to remove unreacted synthetic gene of interest and unreacted synthetic IVT facilitator cassette away from the synthetic product;
transporting the synthetic product to a third one or more reactors in the microfluidic path device and amplifying the synthetic product to generate greater than 1 mM of an
10 amplified DNA template; and
receiving, in a controller for the closed-path system, optical sensor data from one or more sensors of the closed-path system, wherein the controller controls the operation of the closed-path system based on the optical sensor data.
55. An automated method of making a synthetic double-stranded DNA template for *in vitro* transcription using a closed-path system comprising a plurality of fluid depots in sealed fluid communication with a microfluidic path device, the method comprising:
15 transporting, using a first fluid power circuit, a synthetic gene of interest and a synthetic *in vitro* transcription (IVT) facilitator cassette from one or more fluid depots of the plurality of fluid depots into one or more joining reactors of the microfluidic path plate device in a closed fluidic path that is protected from atmospheric contact and joining
20 the synthetic gene of interest to the IVT facilitator cassette to create a synthetic product;
removing, using a second fluid power circuit, unreacted synthetic gene of interest and unreacted synthetic *in vitro* transcription facilitator cassette away from the synthetic product in the microfluidic path plate device;
25 transferring, using a third fluid power circuit, the synthetic product into one or more amplification reactors of the microfluidic path device and amplifying the synthetic product to generate greater than 1 mM of an amplified DNA template; and
receiving, in a controller for the closed-path system, optical sensor data from one or more optical sensors of the closed-path system, wherein the controller controls the first,
30 second and third fluid power circuits based on the optical sensor data and maintains plurality of fluid depots and the microfluidic path devices in a closed-path and sealed environment.
56. An automated method of performing an *in vitro* transcription (IVT) reaction using a system comprising a plurality of fluid depots configured to be secured in sealed fluid communication

with one or more microfluidic path devices, the method comprising transporting reagents between one or more fluid depots of the plurality of fluid depots and a plurality of reactors on the one or more microfluidic devices in a closed fluidic path that is protected from atmospheric contact to perform *in vitro* transcription of a therapeutic mRNA from a template in the one or more microfluidic path devices and to purify the therapeutic polynucleotide.

57. The method of claim 56, wherein the system performs the steps of transporting reagents by deflecting one or more elastic layers within the microfluidic path device.

58. The method of claim 56, further comprising receiving, in a controller for the system, optical sensor data from one or more sensors of the system, wherein the controller controls the operation of the system based on the optical sensor data.

59. The method of claim 56, further comprising pressurizing the fluid depots.

60. The method of claim 56, wherein the microfluidic path devices comprises a microfluidic path plate device seated in the system.

61. The method of claim 56, wherein the method is performed at a site of care.

62. The method of claim 56, wherein the DNA template comprises a double-stranded DNA template of a synthetic gene of interest and a synthetic *in vitro* transcription facilitator cassette.

63. An automated method of performing an *in vitro* transcription (IVT) reaction using a system comprising a plurality of fluid depots configured to be secured in sealed fluid communication with a microfluidic path device, the method comprising:

delivering a DNA template, a polymerase, and nucleotides into one or more IVT reactors of the microfluidic path device from one or more of: a fluid depot of the plurality of fluid depots and a site on the microfluidic path device;

processing, in the one or more IVT reactors, the DNA template and nucleotides to form a therapeutic mRNA; and

transferring the therapeutic mRNA into a one or more purification reactor regions of the microfluidic path device and purifying the therapeutic mRNA by two-dimensional (2D) purification within the one or more purification reactor regions,

wherein the microfluidic path device and the plurality of fluid depots form a closed-path and sealed environment preventing atmospheric exposure.

64. The method of claim 63, further comprising receiving, in a controller for the closed-path system, optical sensor data from one or more sensors of the closed-path system, wherein the controller controls the operation of the closed-path system based on the optical sensor data.
65. The method of claim 63, further comprising pressurizing the fluid depots.
- 5 66. The method of claim 63, wherein the delivering and transporting steps each comprise using one or more fluid power circuits, controlled by a controller, to move the DNA template, polymerase, nucleotides, and therapeutic mRNA material between the plurality of fluid depots and the microfluidic path device or within the microfluidic path device.
67. The method of claim 63, wherein the delivering and transporting steps are performed, under
10 the control of a controller, by deflecting one or more elastic layers within the microfluidic path device to avoid atmospheric contact during the method.
68. The method of claim 56, wherein the microfluidic path devices comprises a microfluidic path plate device seated in the system.
69. The method of claim 63, wherein the method is performed at a site of care.
- 15 70. The method of claim 63, wherein the IVT reactor comprises a pair of connected chambers, each having a liquid-receiving portion and a pressure-receiving portion, wherein the liquid-receiving portion is separated from the pressure-receiving portion by an elastic layer that may be deflected by the pressure-receiving portion to adjust the volume of the liquid receiving portion.
- 20 71. The method of claim 63, wherein the DNA template comprises a double-stranded DNA template of a synthetic gene of interest and a synthetic *in vitro* transcription facilitator cassette.
72. The method of claim 63, further comprising sealing the plurality of fluid depots in fluid communication with a plurality of receiving ports on the microfluidic path device.
- 25 73. An automated method of performing an *in vitro* transcription (IVT) reaction using a system comprising a plurality of fluid depots configured to be secured in sealed fluid communication with a microfluidic path device, the method comprising:
pressurizing the plurality of fluid depots;
delivering, using one or more first fluid power circuits, a DNA template, a polymerase,
30 and nucleotides into one or more IVT reactors of the microfluidic path device in

amounts metered with sub-microliter precision from one or more of: a fluid depot of the plurality of fluid depots and a site on the microfluidic path device; processing, in the one or more IVT reactors, the template material and nucleotides to form a therapeutic mRNA; and

5 transferring, using a second fluid power circuit, the therapeutic mRNA into a one or more purification reactor regions of the microfluidic path device and purifying the therapeutic mRNA by two-dimensional (2D) purification within the one or more purification reactor regions,

10 wherein the microfluidic path device and the plurality of fluid depots form a closed-path and sealed environment preventing atmospheric exposure.

74. A non-transitory computer readable medium embodying instructions for performing an *in vitro* transcription (IVT) reaction, that when executed by a controller of a system comprising a plurality of fluid depots configured to be secured in sealed fluid communication with one or more microfluidic path devices, cause the controller to perform the method of:

15 pneumatically delivering a template material, a polymerase, and nucleotides into a first reactor of a microfluidic path device from a plurality of fluid depots in amounts metered with sub-microliter precision at any time during the reaction;

 processing the template material and nucleotides in the first reactor to form a therapeutic mRNA; and

20 pneumatically transferring the therapeutic mRNA through the microfluidic path device away from the first reactor,

 wherein the first microfluidic path device and the plurality of fluid depots form a closed-path and sealed environment to prevent atmospheric exposure.

75. A method of manufacturing a therapeutic mRNA composition using a system comprising a plurality of fluid depots in sealed fluid communication with one or more microfluidic path plate devices, wherein the one or more microfluidic path plate devices comprise a plurality of reactors, the method comprising:

25 delivering a template precursor material from one or more fluid depots of the plurality of fluid depots to a first reactor of the plurality of reactors and processing the template precursor material to form a DNA template from the template precursor material;

30 transferring the DNA template to a second reactor of the plurality of reactors and processing the DNA template by *in vitro* transcription to form a therapeutic mRNA;

transferring the therapeutic mRNA to a third reactor of the plurality of reactors and
processing the therapeutic mRNA to combine it with a delivery vehicle to form the
therapeutic mRNA composition; and
transferring the therapeutic mRNA composition to a concentrator in fluid communication
5 with the third reactor.

76. The method of claim 75, wherein transferring the therapeutic mRNA to a third reactor
comprises transferring multiple different therapeutic mRNAs with the delivery vehicle to
form the mRNA composition.
77. The method of claim 75, wherein the delivering and transferring steps are performed by one
10 or more fluid power circuits in the system, controlled by a controller.
78. The method of claim 77, wherein the controller controls the fluid power circuits by deflecting
one or more elastic layers within the one or more microfluidic path plate devices.
79. The method of claim 75, wherein the method is performed at a site of care.
80. The method of claim 75, wherein combining the therapeutic mRNA with the delivery vehicle
15 further comprises dialyzing the mRNA therapeutic composition in the one or more
microfluidic path plate devices to purify the mRNA therapeutic composition.
81. The method of claim 75, wherein the delivery vehicle comprises an amphipathic
nanoparticle.
82. The method of claim 81, wherein the amphipathic nanoparticle comprises an amino-lipidated
20 peptoid.
83. The method of claim 75, further comprising purifying the therapeutic mRNA by two-
dimensional (2D) purification within one or more of the plurality of reactors in fluid
communication with the second reactor.
84. The method of claim 75, wherein the method of manufacturing the therapeutic mRNA
25 composition takes 72 hours or less.
85. The method of claim 75, wherein the first reactor of the plurality of reactors is on a first
microfluidic path plate device of the plurality of microfluidic path plate devices and the third
reactor of the plurality of reactors is on a second microfluidic path plate device.

86. The method of claim 75, further comprising receiving, in a controller for the system, optical sensor data from one or more sensors of the system, wherein the controller controls the operation of the closed-path system based at least in part on the optical sensor data.
87. A method of manufacturing a therapeutic mRNA composition using a system comprising a plurality of fluid depots in sealed fluid communication with one or more microfluidic path plate devices, wherein the one or more microfluidic path plate devices comprise a plurality of reactors, the method comprising:
- pressurizing the plurality of fluid depots;
 - controlling a first fluid power circuit to deliver a template precursor material from one or more fluid depots of the plurality of fluid depots to a first reactor of the plurality of reactors with sub-microliter precision and without atmospheric contact;
 - processing the template precursor material to form a DNA template from the template precursor material;
 - controlling a second fluid power circuit to transfer the DNA template to a second reactor of the plurality of reactors with sub-microliter precision and without atmospheric contact;
 - processing the DNA template by *in vitro* transcription to form a therapeutic mRNA;
 - controlling a third fluid power circuit to transfer the therapeutic mRNA to a third reactor of the plurality of reactors with sub-microliter precision and without atmospheric contact;
 - processing the therapeutic mRNA to combine it with a delivery vehicle to form the therapeutic mRNA composition;
 - controlling a third fluid power circuit to transfer the therapeutic mRNA composition to a concentrator in fluid communication with the third reactor; and
 - concentrating the therapeutic mRNA composition.
88. A method of producing a therapeutic polynucleotide composition on-demand, the method comprising:
- receiving, at a local facility, a therapeutic polynucleotide that has been synthesized at a remote facility;
 - formulating the therapeutic polynucleotide composition at the local facility by performing, in an automated system that is protected from atmospheric contact, the steps of:

combining the therapeutic polynucleotide with a delivery vehicle in a microfluidic path device held in the system to form the therapeutic polynucleotide composition,
dialyzing the therapeutic polynucleotide composition in the microfluidic path device; and
providing the therapeutic polynucleotide composition.

89. The method of claim 88, wherein synthesizing the therapeutic polynucleotide comprise synthesizing the therapeutic polynucleotide using a microfluidic system at the remote facility by performing, in a closed fluidic path apparatus that is protected from atmospheric contact, the steps of: forming a synthetic template, performing *in vitro* transcription from the synthetic template to form the therapeutic polynucleotide; and purifying the therapeutic polynucleotide.

90. The method of claim 88 wherein the local facility is a hospital or clinic.

91. The method of claim 88, further comprises concentrating the therapeutic polynucleotide composition.

92. The method of claim 88 further comprising receiving the delivery at the local facility.

93. The method of claim 88, wherein the therapeutic polynucleotide composition comprises an mRNA vaccine.

94. The method of claim 88, wherein formulating the therapeutic polynucleotide comprises using the system, wherein the system comprises a plurality of fluid depots configured to be secured in sealed fluid communication with the microfluidic path device.

95. The method of claim 92, wherein the steps of combining the therapeutic polynucleotide with the delivery vehicle comprises using a first fluid power circuit to deliver the therapeutic polynucleotide and the delivery vehicle from the plurality of fluid depots to one or more reactors of the microfluidic path device with sub-microliter precision and without atmospheric contact.

96. The method of claim 88, wherein formulating the therapeutic polynucleotide composition at the local facility further comprises combining one or more additional therapeutic polynucleotides with the therapeutic polynucleotide and the delivery vehicle.

97. The method of claim 88, wherein the therapeutic polynucleotide is an mRNA, a circular RNA or a self-replicating RNA.
98. The method of claim 88, further comprising storing the therapeutic polynucleotide at the local facility prior to formulating the therapeutic composition.
- 5 99. A method of producing a therapeutic mRNA composition on-demand, the method comprising:
- synthesizing a therapeutic mRNA at a remote facility;
 - transporting the therapeutic mRNA to a local facility;
 - 10 formulating the therapeutic mRNA composition at the local facility by performing, in an automated closed fluidic path apparatus that is protected from atmospheric contact, the steps of:
 - combining the therapeutic mRNA with delivery vehicle in a microfluidic path device to form the therapeutic mRNA composition,
 - dialyzing the therapeutic mRNA composition in the microfluidic path
 - 15 device; and
 - providing the therapeutic mRNA composition.