Claims:

1. A device for applying tension to a nucleic acid sequence, comprising:

one or more fluid channels;

a means of retaining nucleic acid molecules within the one or more fluid channels;

a means for applying a variable and controlled amount of tension to the nucleic acid molecules retained therein, and

further comprising one or more chambers, configured for nucleic acid amplification, replication or template-driven primer extension, for reacting, storing, or introducing reagents, wherein the reagents include nucleic acid primers, nucleotide triphosphates, and polymerase.

2. The device of claim 1, wherein the means for applying tension to the nucleic acid molecules retained in the device comprises first and second surfaces with means for anchoring nucleic acid molecules thereon, and further wherein said first and second surfaces are configured for moving relative to each other.

3. The device of claim 1, wherein the means for applying tension to the nucleic acid molecules comprises at least one surface with means for anchoring nucleic acid molecules thereon, the device further comprising a means for providing a controlled and variable fluid flow over said nucleic acid molecules.

4. The device of claim 3, wherein at least one surface with means for anchoring nucleic acid molecules thereon further comprises passages for fluid flow distributed between the means for anchoring said nucleic acid molecules.

5. The device of claim 3 wherein the means for providing a controlled and variable fluid flow over said nucleic acid molecules is configured to create a velocity gradient in laminar fluid flow.

6. The device of claim 1, wherein the means for applying tension to the nucleic acid molecules retained in the device comprises fluid flow channels configured

to provide a velocity gradient in laminar fluid flow, a stagnation point within a fluid flow, counter propagating fluid flows, or a combination of these.

7. The device of claim 1, wherein the means for applying tension to the nucleic acid molecules retained in the device comprises an array of optical, electrical, or magnetic manipulators configured to manipulate particles bound to the nucleic acid molecules.

8. The device of claim 1, wherein the device is configured to operate isothermally.

9. The device of claim 1 configured for processing nucleic acid molecules, wherein processing includes replicating, sequencing or identifying.

10. The device of claim 1 configured as a microfluidic device for processing nucleic acid molecules, wherein the device is configured to apply tension for denaturing nucleic acid molecules.

11. The device of Claim 1, wherein the means for retaining the nucleic acid molecules includes anchoring means, and the means for applying variable and controlled tension includes an array of movable individually controlled elements or particles that can be manipulated.

12. The device of Claim 11, wherein anchoring means are covalent bonding, antibody-antigen bonding, or streptavidin-biotin bonding.

13. The device of Claims 11, wherein the movable individually controlled elements are piezoelectric elements.

14. The device of Claim 1, wherein the means for applying a variable and controlled amount of tension comprises surfaces arranged to form an array of individually movable elements, each said movable element individually addressable by a control circuit driven by a programmable processor.

15. The device of Claim 14, wherein the control circuit includes a feedback channel that reports force and/or displacement to the processor.

16. The device of Claim 11, wherein the means for applying tension to the nucleic acid molecules comprises at least one surface with means for anchoring nucleic acid molecules thereon, said at least one surface including passages for fluid flow distributed between the means for anchoring said nucleic acid molecules.

17. The device of claim 11, wherein the means for applying tension to the nucleic acid molecules retained in the device comprises optical or magnetic tweezers configured to manipulate particles bound to the nucleic acid molecules.

18. The device of Claim 17, wherein the optical tweezers trap particles with forces generated by optical gradients.

19. The device of Claim 17, further comprising arrays of optical tweezers.

20. The device of Claim 17, further configured to trap particles bound to the nucleic acid molecules in a fluid flow.

21. The device of claim 1, wherein the means for applying tension to the nucleic acid molecules comprises at least one surface having a nucleic acid polymerase attached thereto, the device further comprising a means for providing a controlled and variable fluid flow over said nucleic acid molecules.

22. The device of claim 11, further comprising:

at least one surface having a plurality of support structures thereon, said support structures having means for anchoring nucleic acid molecules; and

a means for providing a controlled and variable fluid flow over said nucleic acid molecules,

wherein the support structures are configured so that fluid can flow between the structures at a controlled rate.

23. The device of claim 11, wherein the means for applying tension to the nucleic acid molecules retained in the device comprises fluid channels configured to create a velocity gradient.

24. The device of Claim 23, wherein the fluid channels are configured to provide for hydrodynamic focusing.

25. The device of Claim 23, wherein the fluid channels are configured to provide for counterpropagating flow.

26. The device of Claim 23, wherein the fluid channels are configured to provide for a T-shaped junction.

27. The device of Claim 11, wherein the means for applying tension to the nucleic acid molecules retained in the device comprises means for applying electric fields to drive fluid flow within the device or means for applying electric fields to the nucleic acid molecules retained in the device.

28. The device of Claim 27, wherein electric field is applied to manipulate conductive particles having nucleic acid molecules attached thereto.

29. A kit for processing a nucleic acid molecule, comprising:

a device of Claim 11; and

particles having nucleic acid polymerization primers attached thereto.

30. A kit for processing a nucleic acid molecule, comprising:

a device of Claim 11; and

reagents useful for amplifying, sequencing or identifying a nucleic acid molecule.

31. The device of Claim 11, wherein the device is handheld.

32. The device of claim 1, wherein the means for applying tension to the nucleic acid molecules retained in the device comprises first and second surfaces with means for anchoring nucleic acid molecules thereon, and further wherein said first and second surfaces are configured for moving relative to each other.

33. The device of claim 1 configured as a microfluidic device for applying tension to nucleic acid molecules, comprising:

a) a substrate;

b) a flow channel disposed within the substrate;

c) an inlet in fluid communication with the flow channel through which a sample of nucleic acid molecules can be introduced into the flow channel, wherein the means for retaining nucleic acid molecules within the device includes,

one or more chambers for reacting, storing, or introducing reagents, wherein the reagents include nucleic acid primers, nucleotide triphosphates, and polymerase.

34. The microfluidic device according to claim 33, wherein the substrate comprises an elastomeric material.

35. The microfluidic device according to claim 33, wherein the flow channel is configured such that a sample introduced into the flow channel can be cycled around the flow channel.

36. The microfluidic device according to claim 33, wherein the microfluidic device further comprises a pump operatively disposed to transport fluid through the channel.

37. The microfluidic device according to claim 33, further configured to retain nucleic acids within the flow channel.

38. The microfluidic device according to claim 33, comprising at least one polymerase molecule immobilized within the flow channel.

39. The microfluidic device according to claim 35, wherein the flow channel is substantially circular.

40. The microfluidic device according to claim 39, wherein the microfluidic device further comprises a pump operatively disposed to transport fluid through the channel.

41. The device of claim 1 configured as a device for sequencing a DNA molecule, comprising:

one or more chambers for reacting, storing, or introducing reagents, said chambers comprising four samples, each sample comprising:

a single-stranded or a double-stranded DNA target sequence,

a DNA polymerase;

a primer complementary to the target sequence;

at least four different nucleoside triphosphates; and

a respective dideoxy nucleoside triphosphate selected from among ddATP, ddCTP, ddGTP, and ddTTP;

wherein sequencing includes the steps:

(a) denaturing the double-stranded DNA target sequence into single-stranded DNA target sequence;

(b) hybridizing the primer to its complementary single-stranded DNA target sequence;

(c) extending the primers to form extension products;

(d) optionally repeating steps (b) through (d) to form further extension products; and

(e) determining the sequence of the DNA target sequence,

wherein tension is applied to one or more strands of DNA target sequence during each cycle of steps (a) through (c).

42. The device of Claim 41, wherein step (e) is performed by detecting the length of the extension product.

43. The device of Claim 42, wherein the dideoxy nucleoside triphosphates are labeled by a detectable chemical moiety.

44. The device of Claim 41, wherein the dideoxy nucleoside triphosphates are labeled by base-specific fluorescent moieties and wherein step (e) is performed by detecting the base-specific fluorescent moiety.

45. The device of Claim 41, wherein the DNA target sequence is amplified prior to performing steps (a) through (e).

46. The device of claim 1 configured for detecting presence of a pathogen in a biological sample, comprising:

one or more chambers for reacting, storing, or introducing reagents for nucleic acid amplification,

a means for probing the amplified nucleic acid for the presence of a nucleotide sequence specifically associated with the pathogen,

wherein detecting includes the steps:

(a) performing a nucleic acid amplification:

(i) denaturing a double-stranded nucleic acid to produce single-stranded template strands;

(ii) annealing at least one primer to the template strands;

(iii) extending the at least one primer by a template-strand-driven polymerase to produce extension products, thereby forming double-stranded nucleic acid molecules; and

(iv) repeating steps (i)-(iii) to amplify the double-stranded nucleic acid, wherein at least one cycle of steps (i)-(iii) comprises applying tension to the nucleic acid, and

(b) probing the amplified nucleic acid for the presence of a nucleotide sequence specifically associated with the pathogen;

wherein pathogen or the nucleotide sequence are detected at concentrations lower than 1000 organisms or polynucleotides/ml.

47. The device of Claim 46, wherein the tension applied is selected from mechanical tension, hydrodynamic tension, or electromagnetic tension, or a combination thereof.

48. The device of Claim 46, wherein pathogen or polynucleotides are detected at concentrations lower than 100 organisms or polynucleotides/ml.

49. The device of Claim 46, wherein pathogen or polynucleotides are detected at concentrations lower than 50 organisms or polynucleotides/ml.

50. The device of Claim 46, wherein pathogen or polynucleotides are detected at concentrations lower than 25 organisms or polynucleotides/ml.

51. The device of Claim 46, wherein pathogen or polynucleotides are detected at concentrations lower than 5 organisms or polynucleotides/ml.

52. The device of Claim 46, wherein pathogen or polynucleotides are detected at a concentration of 1 organism or polynucleotides/ml.

53. The device of claim 1 configured for performing nucleic acid amplification, comprising:
one or more chambers for reacting, storing, or introducing reagents for nucleic acid amplification,
wherein amplification includes the steps:
(a) denaturing a double-stranded nucleic acid to produce single-stranded template strands;
(b) annealing at least one primer to the template strands;
(c) extending the at least one primer by a template-strand-driven polymerase to produce extension products, thereby forming double-stranded nucleic acid molecules; and
(d) repeating steps (a)-(c) to amplify the double-stranded nucleic acid, wherein at least one of the double-stranded nucleic acid molecules produced in step (c) in a cycle of steps (a)-(c) is denatured in step (a) in a subsequent cycle of steps (a)-(c), so that at least one extension product formed in step (c) is used as a template strand in a subsequent cycle of steps (a)-(d), and
wherein at least one cycle of steps (a)-(c) comprises applying tension to the template strand, and

wherein the tension is adjusted according to the position of the polymerase along the template strand.

54. The device of Claim 53, wherein the position of the polymerase along the template strand is monitored.

55. The device of Claim 53, wherein the position of the polymerase along the template strand is computed.

56. The device of Claim 53, wherein the position of the polymerase corresponds to a location of a difficult sequence.

57. The device of claim 1 configured for detecting presence of at least one specific nucleic acid sequence in a sample, comprising:
one or more chambers for reacting, storing, or introducing reagents for nucleic acid amplification,
wherein amplification includes the steps:
(a) performing a nucleic acid amplification:
(i) denaturing a double-stranded nucleic acid to produce single-stranded template strands;
(ii) annealing at least one primer to the template strands;
(iii) extending the at least one primer by a template-strand-driven polymerase to produce extension products, thereby forming double-stranded nucleic acid molecules; and
(iv) repeating steps (i)-(iii) to amplify the double-stranded nucleic acid, thereby producing amplified nucleic acid,
wherein at least one cycle of steps (i)-(iii) comprises applying tension to the nucleic acid, and
(b) contacting the amplified nucleic acid with at least one probe; and

(c) detecting whether the specific nucleic sequence is in the sample by observing the presence or absence of the probe bound to the amplified nucleic acid.

58. The device of claim 1 configured for distinguishing between at least two specific nucleic acid sequences in a sample, comprising:

one or more chambers for reacting, storing, or introducing reagents for nucleic acid amplification,

wherein the amplification includes the steps:

(a) performing a nucleic acid amplification:

(i) denaturing a double-stranded nucleic acid to produce single-stranded template strands;

(ii) annealing at least one primer to the template strands;

(iii) extending the at least one primer by a template-strand-driven polymerase to produce extension products, thereby forming double-stranded nucleic acid molecules; and

(iv) repeating steps (i)-(iii) to amplify the double-stranded nucleic acid, thereby producing amplified nucleic acid,

wherein at least one cycle of steps (i)-(iii) comprises applying tension to the nucleic acid, and

(b) contacting the amplified nucleic acid with a plurality of probes; and

(c) detecting which of a plurality of probes is bound to the amplified nucleic acid, thereby distinguishing between at least two specific nucleic acid sequences.

59. The device of claim 1 configured for amplification or detection of sequences encoded on RNA, comprising:

one or more chambers for reacting, storing, or introducing reagents for nucleic acid amplification,

wherein amplification includes the steps:

(a) isolating RNA from a sample;

(b) performing reverse transcription thereby obtaining a corresponding cDNA template;

(c) amplifying the corresponding cDNA template by a method, including:

(i) optionally denaturing a double-stranded cDNA template to produce at least one single-stranded cDNA template strand;

(ii) annealing at least one primer to the at least one cDNA template strand;

(iii) extending the at least one primer by a template-strand-driven polymerase to produce extension products, thereby forming double-stranded nucleic acid molecules; and

(iv) repeating steps (i)-(iii) to amplify the double-stranded nucleic acid, thereby amplifying or detecting sequences encoded on RNA,

wherein at least one cycle of steps (i)-(iii) comprises applying tension to the nucleic acid.

60. A device of claim 1, wherein at least one of the one or more chambers contain reagents for template-driven primer extension.

61. A device of any one of claims 41, 46, 53, 57, 58 and 59, wherein tension is applied during template-driven primer extension.

WHAT IS CLAIM IS:

1. A device for applying tension to a nucleic acid sequence, comprising:

one or more fluid channels for directing fluid flow within the device;

a means of retaining nucleic acid molecules within the one or more fluid channels;

a mechanism for applying tension, that tends to stretch the nucleic acid molecule, to the nucleic acid molecules retained therein during template-driven primer extension, replication or at least one cycle of amplification,

further comprising one or more chambers, configured for nucleic acid amplification, replication or template-driven primer extension, for reacting, storing, or introducing reagents, wherein the reagents include nucleic acid primers, nucleotide triphosphates, and polymerase.

2. The device of claim 1, wherein the mechanism for applying tension to the nucleic acid molecules retained in the device comprises first and second surfaces with means for anchoring nucleic acid molecules thereon, and further wherein said first and second surfaces are configured for moving relative to each other.

3. The device of claim 1, wherein the mechanism for applying tension to the nucleic acid molecules comprises at least one surface with means for anchoring nucleic acid molecules thereon, the device further comprising a mechanism for providing a fluid flow over said nucleic acid molecules.

4. The device of claim 3, wherein at least one surface with means for anchoring nucleic acid molecules thereon further comprises passages for fluid flow distributed between the means for anchoring said nucleic acid molecules.

5. The device of claim 3 wherein the mechanism for providing a fluid flow over said nucleic acid molecules is configured to create a velocity gradient in laminar fluid flow.

6. The device of claim 1, wherein the mechanism for applying tension to the nucleic acid molecules retained in the device comprises fluid flow channels configured to provide a velocity gradient in laminar fluid flow, a stagnation point within a fluid flow, counter propagating fluid flows, or a combination of these.

7. The device of claim 1, wherein the mechanism for applying tension to the nucleic acid molecules retained in the device comprises an array of optical, electrical, or magnetic manipulators configured to manipulate particles bound to the nucleic acid molecules.

8. The device of claim 1, wherein the device is configured to operate isothermally.

9. The device of claim 1 configured for processing nucleic acid molecules, wherein processing includes replicating, sequencing or identifying.

10. The device of claim 1 configured as a microfluidic device for processing nucleic acid molecules, wherein the device is configured to apply tension for denaturing nucleic acid molecules.

11. The device of Claim 1, wherein the means for retaining the nucleic acid molecules includes anchoring means, and the mechanism for applying tension includes an array of movable individually controlled elements or particles.

12. The device of Claim 11, wherein anchoring means are covalent bonding, antibody-antigen bonding, or streptavidin-biotin bonding.

13. The device of Claims 11, wherein the movable individually controlled elements are piezoelectric elements.

14. The device of Claim 1, wherein the mechanism for applying tension comprises surfaces arranged to form an array of individually movable elements, each said movable element individually addressable by a control circuit driven by a programmable processor.

15. The device of Claim 14, wherein the control circuit includes a feedback channel that reports force and/or displacement to the processor.

16. The device of claim 11, wherein the mechanism for applying tension to the nucleic acid molecules retained in the device comprises optical or magnetic tweezers configured to manipulate particles bound to the nucleic acid molecules.

17. The device of Claim 16, wherein the optical tweezers trap particles with forces generated by optical gradients.

18. The device of Claim 16, further comprising arrays of optical tweezers.

19. The device of Claim 16, further configured to trap particles bound to the nucleic acid molecules in a fluid flow.

20. The device of claim 1, wherein the mechanism for applying tension to the nucleic acid molecules comprises at least one surface having a nucleic acid polymerase attached thereto, the device further comprising a mechanism for providing a fluid flow over said nucleic acid molecules.

21. The device of claim 11, further comprising:

at least one surface having a plurality of support structures thereon, said support structures having means for anchoring nucleic acid molecules; and

a mechanism for providing a fluid flow over said nucleic acid molecules,

wherein the support structures are configured so that fluid can flow between the structures at a controlled rate.

22. The device of claim 11, wherein the mechanism for applying tension to the nucleic acid molecules retained in the device comprises fluid channels configured to create a velocity gradient.

23. The device of Claim 22, wherein the fluid channels are configured to provide for hydrodynamic focusing.

24. The device of Claim 22, wherein the fluid channels are configured to provide for counterpropagating flow.

25. The device of Claim 22, wherein the fluid channels are configured to provide for a T-shaped junction.

26. The device of Claim 11, wherein the mechanism for applying tension to the nucleic acid molecules retained in the device comprises mechanism for applying electric fields to drive fluid flow within the device or mechanism for applying electric fields to the nucleic acid molecules retained in the device.

27. The device of Claim 26, wherein electric field is applied to conductive particles having nucleic acid molecules attached thereto.

28. A kit for processing a nucleic acid molecule, comprising:

a device of Claim 11; and

particles having nucleic acid polymerization primers attached thereto.

29. A kit for processing a nucleic acid molecule, comprising:

a device of Claim 11; and

reagents useful for amplifying, sequencing or identifying a nucleic acid molecule.

30. The device of Claim 11, wherein the device is handheld.

31. The device of claim 1, wherein the mechanism for applying tension to the nucleic acid molecules retained in the device comprises first and second surfaces with means for anchoring nucleic acid molecules thereon, and further wherein said first and second surfaces are configured for moving relative to each other.

32. The device of claim 1 configured as a microfluidic device for applying tension to nucleic acid molecules, comprising:

a) a substrate;

b) a flow channel disposed within the substrate;

c) an inlet in fluid communication with the flow channel through which a sample of nucleic acid molecules can be introduced into the flow channel, wherein the means for retaining nucleic acid molecules within the device includes,

one or more chambers for reacting, storing, or introducing reagents, wherein the reagents include nucleic acid primers, nucleotide triphosphates, and polymerase.

33. The microfluidic device according to claim 32, wherein the substrate comprises an elastomeric material.

34. The microfluidic device according to claim 32, wherein the flow channel is configured such that a sample introduced into the flow channel can be cycled around the flow channel.

35. The microfluidic device according to claim 32, wherein the microfluidic device further comprises a pump operatively disposed to transport fluid through the channel.

36. The microfluidic device according to claim 32, further configured to retain nucleic acids within the flow channel.

37. The microfluidic device according to claim 32, comprising at least one polymerase molecule immobilized within the flow channel.

38. The microfluidic device according to claim 34, wherein the flow channel is substantially circular.

39. The microfluidic device according to claim 38, wherein the microfluidic device further comprises a pump operatively disposed to transport fluid through the channel.

40. The device of claim 1 configured as a device for sequencing a DNA molecule, comprising:

one or more chambers for reacting, storing, or introducing reagents, said chambers comprising four samples, each sample comprising:

a single-stranded or a double-stranded DNA target sequence,

a DNA polymerase;

a primer complementary to the target sequence;

at least four different nucleoside triphosphates; and

a respective dideoxy nucleoside triphosphate selected from among ddATP, ddCTP, ddGTP, and ddTTP.

41. The device of Claim 40, wherein the dideoxy nucleoside triphosphates are labeled by a detectable chemical moiety.

42. The device of Claim 40, wherein the dideoxy nucleoside triphosphates are labeled by base-specific fluorescent moieties.

43. The device of claim 1 configured for detecting presence of a pathogen in a biological sample, comprising:

a means for probing the amplified nucleic acid for the presence of a nucleotide sequence specifically associated with the pathogen.

44. The device of Claim 1, wherein the mechanism for applying tension is selected from a mechanism for applying mechanical tension, hydrodynamic tension, or electrodynamic tension, or a combination thereof.

45. A device of claim 1, wherein at least one of the one or more chambers contain reagents for template-driven primer extension.

46. A device of any one of claims 40 and 43, wherein tension is applied during template-driven primer extension.