What is claimed is:

1. A method of sequencing a polynucleotide using a nanopore comprising a first side, a second side, and an aperture extending through the first and second sides, the method comprising:

(a) disposing a polynucleotide through the aperture of the nanopore such that a 3' end of the polynucleotide is on the first side of the nanopore, and a 5' end of the polynucleotide is on the second side of the nanopore;

(b) forming a duplex with the polynucleotide on the first side of the nanopore, the duplex including a 3' end;

(c) extending the duplex on the first side of the nanopore by adding a first nucleotide to the 3' end of the duplex;

(d) applying a force disposing the 3’ end of the extended duplex within the aperture, and while applying the force:

inhibiting, using the nanopore, translocation of the 3' end of the extended duplex to the second side of the nanopore; and

measuring a value of an electrical property of the 3' end of the extended duplex and a single-stranded portion of the polynucleotide,

wherein the value measured in operation (d) is at least based on M nucleotides of the single-stranded portion of the polynucleotide and D pairs of hybridized nucleotides of the extended duplex, wherein M is greater than or equal to two, and wherein D is greater than or equal to one; and

(e) identifying the first nucleotide using the value measured in operation (d).

2. The method of claim 1, wherein the value measured in operation (d) comprises an electrical current, ionic current, electrical resistance, or electrical voltage drop across the nanopore, or wherein the value measured in operation (d) comprises noise of an electrical current, ionic current, electrical resistance, or electrical voltage drop across the nanopore.

3. The method of claim 1 or claim 2, wherein M is greater than or equal to three, or wherein D is greater than or equal to two.

4. The method of any one of claims 1 to 3, wherein at least one of the M nucleotides of the single-stranded portion comprises a modified base, the method comprising

identifying the modified base using the value measured in operation (d), optionally wherein the modified base comprises a methylated base.

5. The method of any one of claims 1 to 4, further comprising inhibiting addition of another nucleotide to the 3' end of the extended duplex while the force is applied in operation (d), optionally wherein the nanopore inhibits the addition of another nucleotide.

6. The method of any one of claims 1 to 5, further comprising:

(f) applying a modified force again disposing the 3′ end of the extended duplex within the aperture, and while applying the modified force:

inhibiting, using the nanopore, translocation of the 3' end of the extended duplex to the second side of the nanopore; and

measuring a value of an electrical property of the 3' end of the extended duplex and a single-stranded portion of the polynucleotide; and

(g) identifying the first nucleotide using the value measured in operation (f).

7. The method of claim 6, wherein the modified force of operation (f) disposes the 3′ end of the extended duplex at a different location relative to the nanopore than does the force of operation (d).

8. The method of claim 6 or claim 7, wherein the modified force of operation (f) is applied before another nucleotide is added, or wherein the modified force of operation (f) is applied while resequencing the polynucleotide.

9. The method of any one of claims 6 to 8, wherein the first nucleotide is identified using a comparison of the value measured in operation (d) to the value measured in operation (f), or wherein a trained neural network identifies the first nucleotide based upon input of the value measured in operation (d) and the value measured in operation (f).

10. The method of any one of claims 1 to 9, wherein the first nucleotide is added using a polymerase in contact with the 3' end of the duplex, optionally further comprising reversibly inhibiting the polymerase from adding a second nucleotide to the 3' end of the extended duplex, optionally wherein a blocking moiety reversibly inhibits the polymerase from

adding the second nucleotide to the 3' end of the extended duplex, optionally further comprising removing the blocking moiety to allow the polymerase to add the second nucleotide to the 3' end of the extended duplex.

12. The method of claim 11, wherein the force applied in (d) removes the polymerase from contact with the 3' end of the extended duplex, or further comprising applying a force which is different than the force applied in (d) to remove the polymerase from contact with the 3' end of the extended duplex.

13. The method of claim 11 or claim 12, wherein the polymerase comprises a DNA polymerase, or wherein the polymerase comprises an RNA polymerase, or wherein the polymerase comprises a reverse transcriptase.

14. The method of any one of claims 1 to 13, wherein the extended duplex comprises one or more nucleotide analogues, optionally wherein the one or more nucleotide analogues enhance stability of the extended duplex relative to a natural nucleotide, or optionally wherein the one or more nucleotide analogues comprise one or more locked nucleic acids (LNA), one or more 2'-methoxy (2'-OMe) nucleotides, or one or more 2'-fluorinated (2'-F) nucleotides.

15. The method of claim 14, wherein the one or more nucleotide analogues alter the value of the electrical property relative to a natural nucleotide, or wherein the first nucleotide comprises one of the one or more nucleotide analogues, optionally wherein the one or more nucleotide analogues comprise a 2’ modification or a base modification.

16. The method of any one of claims 1 to 15, wherein the force applied in operation (d) is insufficiently strong to cause dissociation of the extended duplex, or wherein the force applied in operation (d) comprises a first voltage, or wherein operations (b) and (c) are performed in the absence of the force applied in operation (d), or wherein operation (c) is performed in the presence of a force that opposes the force applied in operation (d).

17. The method of any one of claims 1 to 16, wherein a first locking structure is coupled to the 3'-end of the polynucleotide on the first side of the nanopore, the first locking

structure inhibiting translocation of the 3'-end of the polynucleotide to the second side of the nanopore through the aperture, optionally wherein the first locking structure is removable, or wherein a second locking structure is coupled to the 5'-end of the polynucleotide on the second side of the nanopore, the second locking structure inhibiting translocation of the 5'-end of the polynucleotide to the first side of the nanopore through the aperture, optionally wherein the second locking structure is removable.

18. The method of any one of claims 1 to 17, further comprising, after operation (d):

dissociating the extended duplex from the polynucleotide; and

forming a new duplex with the polynucleotide on the first side of the nanopore, the new duplex including a new 3' end.

19. The method of claim 18, further comprising, after forming the new duplex, repeating operations (c) through (e) to resequence the polynucleotide.

20. The method of claim 19, further comprising generating a consensus read using the identification in operation (e) from sequencing the polynucleotide and the identification in operation (e) from resequencing the polynucleotide.

21. The method of claim 19 or claim 20, wherein the polynucleotide is resequenced responsive to sequencing of the polynucleotide being substantially complete, or wherein the polynucleotide is resequenced as a remedial action.

22. The method of any one of claims 1 to 21, wherein operation (a) comprises:

contacting the nanopore with the polynucleotide hybridized to a substantially complementary polynucleotide; and

applying a force dehybridizing the substantially complementary polynucleotide from the polynucleotide.

23. The method of any one of claims 1 to 22, wherein the nanopore comprises a solid-state nanopore or a biological nanopore, optionally wherein the biological nanopore comprises MspA.

24. The method of any one of claims 1 to 23, wherein the polynucleotide comprises RNA or DNA.

25. The method of any one of claims 1 to 24, wherein the extended duplex comprises a primer hybridized to the polynucleotide.

26. A sequencing system, comprising:

a nanopore comprising a first side, a second side, and an aperture extending through the first and second sides;

a polynucleotide disposed through the aperture of the nanopore such that a 3' end of the polynucleotide is on the first side of the nanopore, and a 5' end of the polynucleotide is on the second side of the nanopore;

a duplex with the polynucleotide disposed on the first side of the nanopore, the duplex including a 3' end at which a first nucleotide is disposed; and

circuitry configured to:

apply a force disposing the 3′ end of the duplex within the aperture;

measure a value of an electrical property of the 3' end of the duplex and a single-stranded portion of the polynucleotide while applying the force,

wherein the measured value is at least based on M nucleotides of the single-stranded portion of the polynucleotide and D pairs of hybridized nucleotides of the extended duplex, wherein M is greater than or equal to two, and wherein D is greater than or equal to one; and

identify the first nucleotide using the measured value,

wherein the nanopore inhibits translocation of the 3' end of the duplex to the second side of the nanopore while the force is applied.

27. The system of claim 26, wherein the value measured by the circuitry comprises an electrical current, ionic current, electrical resistance, or electrical voltage drop across the nanopore, or wherein the value measured by the circuitry comprises noise of an electrical current, ionic current, electrical resistance, or electrical voltage drop across the nanopore.

28. The system of claim 26 or claim 27, wherein M is greater than or equal to three, or wherein D is greater than or equal to two.

29. The system of any one of claims 26 to 28, wherein at least one of the M nucleotides of the single-stranded portion comprises a modified base, the circuitry being configured to identify the modified base using the value measured by the circuitry, optionally wherein the modified base comprises a methylated base.

30. The system of any one of claims 26 to 29, wherein an addition of another nucleotide to the 3' end of the duplex is inhibited while the force is applied, optionally wherein the nanopore inhibits the addition of another nucleotide.

31. The system of any one of claims 26 to 30, wherein the circuitry further is configured to:

apply a modified force again disposing the 3′ end of the duplex within the aperture;

measure a value of an electrical property of the 3' end of the duplex and a single-stranded portion of the polynucleotide while applying the modified force; and

identify the first nucleotide using the measured value,

wherein the nanopore inhibits translocation of the 3' end of the duplex to the second side of the nanopore while the modified force is applied.

32. The system of claim 31, wherein the modified force of disposes the 3′ end of the extended duplex at a different location relative to the nanopore than does the force.

33. The system of claim 31 or claim 32, wherein the modified force is applied before another nucleotide is added, or wherein the modified force is applied while resequencing the polynucleotide.

34. The system of any one of claims 31 to 33, wherein the first nucleotide is identified using a comparison of the value measured under the force to the value measured under the modified force, or wherein a trained neural network identifies the first nucleotide based upon input of the value measured under the force and the value measured under the modified force.

35. The system of any one of claims 26 to 34, further comprising a polymerase in

contact with the 3' end of the duplex and adding the first nucleotide, optionally wherein the polymerase is reversibly inhibited from adding a second nucleotide to the 3' end of the duplex, optionally further comprising a blocking moiety reversibly inhibiting the polymerase from adding the second nucleotide to the 3' end of the duplex, optionally wherein the blocking moiety is removable to allow the polymerase to add the second nucleotide to the 3' end of the duplex.

36. The system of claim 33 or claim 34, wherein the force removes the polymerase from contact with the 3' end of the duplex, or wherein the circuitry further is configured to apply a force to remove the polymerase from contact with the 3' end of the duplex, wherein the force used to remove the polymerase is different than the force used to dispose the 3′ end of the duplex within the aperture.

37. The system of claim 35 or claim 36, wherein the polymerase comprises a DNA polymerase, or wherein the polymerase comprises an RNA polymerase, or wherein the polymerase comprises a reverse transcriptase.

38. The system of any one of claims 26 to 39, wherein the duplex comprises one or more nucleotide analogues, optionally wherein the one or more nucleotide analogues enhance stability of the duplex relative to a natural nucleotide, or wherein the one or more nucleotide analogues comprise one or more locked nucleic acids (LNA), or one or more 2'-methoxy (2'-OMe) nucleotides, or one or more 2'-fluorinated (2'-F) nucleotides.

39. The system of claim 38, wherein the one or more nucleotide analogues alter the value of the electrical property relative to a natural nucleotide, or wherein the first nucleotide comprises one of the one or more nucleotide analogues, optionally wherein the one or more nucleotide analogues comprise a 2’ modification or a base modification.

40. The system of any one of claims 26 to 39, wherein the force used to dispose the 3′ end of the duplex within the aperture is insufficiently strong to cause dissociation of the duplex, or wherein the force used to dispose the 3′ end of the duplex within the aperture comprises a first voltage.

41. The system of any one of claims 26 to 40, wherein the polynucleotide is disposed through the aperture and the duplex is disposed on the first side of the nanopore in the absence of the force used to dispose the 3′ end of the duplex within the aperture, or wherein the circuitry is configured to apply a force that opposes the force used to dispose the 3′ end of the duplex within the aperture.

42. The system of any one of claims 26 to 41, wherein a first locking structure is coupled to the 3'-end of the polynucleotide on the first side of the nanopore, the first locking structure inhibiting translocation of the 3'-end of the polynucleotide to the second side of the nanopore through the aperture, optionally wherein the first locking structure is removable, or wherein a second locking structure is coupled to the 5'-end of the polynucleotide on the second side of the nanopore, the second locking structure inhibiting translocation of the 5'-end of the polynucleotide to the first side of the nanopore through the aperture, optionally wherein the second locking structure is removable.

43. The system of any one of claims 26 to 42, wherein the circuitry is configured to, after applying the force used to dispose the 3′ end of the duplex within the aperture, dissociate the duplex from the polynucleotide and to form a new duplex.

44. The system of claim 43, wherein the circuitry is configured, after forming the new duplex, to resequence the polynucleotide.

45. The system of claim 44, wherein the circuitry is configured to generate a consensus read using the identification from sequencing the polynucleotide and the identification from resequencing the polynucleotide.

46. The system of claim 43 or claim 44, wherein the circuitry is configured to resequence the polynucleotide responsive to sequencing of the polynucleotide being substantially complete, or wherein the circuitry is configured to resequence the polynucleotide as a remedial action.

47. The system of any one of claims 26 to 46, wherein the circuitry is configured to apply a force dehybridizing a substantially complementary polynucleotide from the

polynucleotide so as to dispose the polynucleotide through the aperture of the nanopore.

48. The system of any one of claims 26 to 47, wherein the nanopore comprises a solid-state nanopore or a biological nanopore, optionally wherein the biological nanopore comprises MspA.

49. The system of any one of claims 26 to 48, wherein the polynucleotide comprises RNA or DNA.

50. The system of any one of claims 26 to 49, wherein the duplex comprises a primer hybridized to the polynucleotide.