

WHAT IS CLAIMED:

1. A composition including:

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

5 a plurality of nucleotides, wherein each of the nucleotides comprises an elongated tag;

first and second polynucleotides, the first polynucleotide being complementary to the second polynucleotide;

10 a polymerase disposed adjacent to the first side of the nanopore, the polymerase configured to add nucleotides of the plurality of nucleotides to the first polynucleotide based on a sequence of the second polynucleotide;

a permanent tether including a head region, a tail region, and an elongated body disposed therebetween, the head region being anchored to the polymerase, wherein the elongated body occurs in the aperture of the nanopore;

15 a first moiety disposed on the elongated body, wherein the first moiety is configured to bind to the elongated tag of a first nucleotide upon which the polymerase is acting; and

20 a reporter region disposed on the elongated body, wherein the reporter region is configured to indicate when the first nucleotide is complementary or is not complementary to a next nucleotide in the sequence of the second polynucleotide.

25 2. The composition of claim 1, the first moiety being configured to generate a first signal state responsive to the polymerase acting upon the first nucleotide, the first nucleotide being identifiable based on the first signal state.

3. The composition of claim 2, wherein the polymerase acting upon the first nucleotide comprises the polymerase binding the first nucleotide, or the first signal state including an electrical or optical signal, or the reporter region being configured to generate a second signal state, it being detectable based upon the second signal state whether the first nucleotide is complementary or is not complementary to the next nucleotide of the second polynucleotide.

4. The composition of claim 3, the reporter region being configured to generate the second signal state responsive to the polymerase successfully incorporating the first nucleotide into the first polynucleotide; preferably the reporter region being configured to generate the second signal state responsive to release of
5 pyrophosphate responsive to the polymerase successfully incorporating the first nucleotide into the first polynucleotide.
5. The composition of claim 4, the polymerase being modified so as to delay release of the pyrophosphate responsive to incorporation of the first nucleotide into
10 the first polynucleotide.
6. The composition of claim 5, wherein the polymerase comprises a modified recombinant Φ 29, B103, GA-1, PZA, Φ 15, BS32, M2Y, Nf, G1, Cp-1, PRD1, PZE, SF5, Cp-5, Cp-7, PR4, PR5, PR722, or L17 polymerase; preferably wherein the
15 polymerase comprises a modified recombinant Φ 29 DNA polymerase having at least one amino acid substitution or combination of substitutions selected from the group consisting of: an amino acid substitution at position 484, an amino acid substitution at position 198, and an amino acid substitution at position 381; more preferably wherein the polymerase comprises a modified recombinant Φ 29 DNA polymerase
20 having at least one amino acid substitution or combination of substitutions selected from the group consisting of E375Y, K512Y, T368F, A484E, A484Y, N387L, T372Q, T372L, K478Y, I370W, F198W, and L381A.
7. The composition of claim 3, the reporter region being configured to generate
25 the second signal state responsive to a conformational change of the polymerase.
8. The composition of claim 7, a magnitude or a time duration of the conformational change of the polymerase being responsive to the first nucleotide being complementary or not complementary to the next nucleotide of the second
30 polynucleotide, a magnitude or a time duration, or both, of the second signal state being based upon the conformational change of the polymerase.

9. The composition of claim 3, wherein the second signal state includes an electrical signal, or an optical signal.
10. The composition of claim 1, wherein the elongated tag comprises a first nucleotide sequence and the first moiety comprises a second nucleotide sequence that is complementary to the first nucleotide sequence.
11. A system including the composition of claim 10, and further including measurement circuitry configured to measure a first current or flux state through the aperture; preferably wherein the first current or flux state is based on the elongated tag, the first nucleotide being identifiable based on the first current or flux state.
12. The composition of claim 11, the measurement circuitry further being configured to measure a second current or flux state through the aperture; preferably wherein the second current or flux state is based on a position of the reporter region within the aperture, it being determinable based on the second current or flux state whether the first nucleotide is complementary or is not complementary to the next nucleotide in the second polynucleotide.
13. A method including:
- (a) providing a nanopore including a first side, a second side, and an aperture extending through the first and second sides;
 - (b) providing a plurality of nucleotides, wherein each of the nucleotides comprises an elongated tag;
 - (c) providing first and second polynucleotides, the first polynucleotide being complementary to the second polynucleotide;
 - (d) providing a polymerase disposed adjacent to the first side of the nanopore, the polymerase configured to add nucleotides of the plurality of nucleotides to the first polynucleotide based on a sequence of the second polynucleotide, wherein the polymerase is anchored to a permanent tether

including a head region, a tail region, and an elongated body disposed therebetween, the elongated body occurring in the aperture of the nanopore;

(e) determining that a first nucleotide is being acted upon by the polymerase based on binding of the elongated tag to a first moiety disposed on the elongated body; and

(f) with a reporter region disposed on the elongated body, indicating when the first nucleotide is complementary or is not complementary to a next nucleotide in the sequence of the second polynucleotide.

10 14. The method of claim 13, further comprising

(g) determining that a subsequent nucleotide is being acted upon by the polymerase based on binding of the elongated tag to the first moiety disposed on the elongated body; and

15 (h) with a reporter region disposed on the elongated body, indicating when the subsequent nucleotide is complementary or is not complementary to a nucleotide that is subsequent to the next nucleotide in the sequence of the second polynucleotide.

20 15. The method of claim 14, further comprising repeating steps (g) and (h) for a plurality of subsequent nucleotides that are complementary or not complementary to a plurality of nucleotides that are subsequent to the next nucleotide.