

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 generate a composite electrical signal of more than one signal state, a first signal state of the composite signal being generated responsive to the polymerase acting upon the first nucleotide based on the first moiety binding to the elongated tag of the first nucleotide to provide information about the identity of the first nucleotide and a second signal state of the composite signal being generated responsive to a conformational change of the polymerase to indicate whether the first nucleotide is complementary or is not complementary to a next nucleotide in the sequence of the second polynucleotide.

2. The composition according to claim 1, wherein the first moiety is configured such that the binding between the first moiety and the first elongated tag of the first nucleotide causes the reporter region to become disposed at a location with the aperture that results in the first signal state through the aperture which is unique and based upon which the first nucleotide can be identified.

3. The composition according to claim 1 or 2, wherein the first moiety comprises a first oligonucleotide.
4. The composition according to claim 3, wherein the elongated tag of each of the plurality of nucleotides comprises an oligonucleotide that is complementary to, and hybridize with, the first oligonucleotide.
5. The composition according to claim 4, wherein each nucleotide of the plurality of nucleotides includes a oligonucleotide moiety corresponding to the type of nucleotide.
6. The composition according to claim 5, wherein the binding between the first moiety and the first elongated tag of the first nucleotide induces a conformational change in the permanent tether that moves the reporter region a distance that corresponds to the nucleotide being acted upon by the polymerase.
7. The composition according to any one of the preceding claims, wherein the elongated tag corresponds to the type of nucleotide.
8. The composition according to any one of the preceding claims, wherein the composite signal state is generated based on a position of the reporter region within the aperture.
9. The composition according to claim 8, wherein the position of the reporter region depends on the conformational state of the polymerase.
10. The composition according to any one of the preceding claims, wherein the conformation of the polymerase is based upon whether the first nucleotide is a match or a mismatch to the next nucleotide in the sequence of the second polynucleotide.
11. A system including any one of claims 1 to 10, and further including measurement circuitry configured to measure the composite signal through the aperture.

12. The system according to claim 11, wherein the measurement circuitry is configured to detect the position of the reporter region.

13. A method including:

5 (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;

10 (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, and a reporter region disposed on the elongated body;

15 (e) the reporter region generating a composite electrical signal of more than one signal state, a first signal state of the composite signal being generated responsive to the polymerase acting upon a first nucleotide based on the binding of the elongated tag to a first moiety disposed on the elongated body, a second signal state of the composite signal being generated responsive to a conformational change of the polymerase;

20 (f) determining, based on the composite signal, the identity of the first nucleotide; and

(f) determining, based on the composite signal, whether the first nucleotide is complementary or is not complementary to a next nucleotide in the sequence of the second polynucleotide.

14. The method of claim 13, wherein the step of determining the identity of the first nucleotide being acted upon by the polymerase is based on the first signal state of the composite signal.

15. The method of claim 14, wherein the step of determining whether the first nucleotide is complementary or is not complementary to a next nucleotide in the

sequence of the second polynucleotide is based on the second signal state of the composite signal.