CLAIMS

1. A method of detecting or quantifying a sense and an antisense strand of an oligonucleotide duplex in a sample, the method comprising:

(a) contacting the sample with a composition comprising a set of probes, wherein the set of probes comprises:

(i) a sense probe comprising a first single stranded oligonucleotide tag that is complementary to at least a portion of a first capture oligonucleotide immobilized on a support surface, a sense binding portion capable of hybridizing to a nucleotide sequence of the sense strand of the oligonucleotide duplex, and a first label; and

(ii) an antisense probe comprising a second single stranded oligonucleotide tag that is complementary to at least a portion of a second capture oligonucleotide immobilized on the support surface, an antisense binding portion capable of hybridizing to a nucleotide sequence of the antisense strand of the oligonucleotide duplex, and a second label,

wherein the sense binding portion of the sense probe has a sense binding length that is shorter than a sense strand length of the sense strand, and

wherein the antisense binding portion of the antisense strand has an antisense binding length that is shorter than an antisense strand length of the antisense strand; and

(b) incubating the probes with the sample to form a hybridization mixture comprising hybridization complexes comprising:

(i) a sense complex comprising the sense probe hybridized with the sense strand of the oligonucleotide duplex; and

(ii) an antisense complex comprising the antisense probe hybridized with the antisense strand of the oligonucleotide duplex; and

(c) contacting the support surface with the hybridization mixture in which the first and second oligonucleotide tags of the sense and antisense probes hybridize to the first and second capture oligonucleotides immobilized on the support surface and contacting the hybridization complexes in the hybridization mixture with a single-strand specific nuclease; and

(d) detecting or quantifying the sense and antisense strands of the oligonucleotide duplex based on the presence of the label on the support surface.

2. The method of claim 1, wherein (c) comprises:

(i) contacting the support surface with the hybridization mixture under conditions in which the first and second oligonucleotide tags of the hybridization complexes hybridize to the first and second capture oligonucleotides on the support surface to immobilize the hybridization complexes on the support surface; and

(ii) contacting the immobilized hybridization complexes with a single-strand specific nuclease; or

(i) contacting the hybridization mixture with a single-strand specific nuclease to form a reaction mixture; and

(ii) contacting the support surface with the reaction mixture of (i) under conditions in which the first and second oligonucleotide tags of the hybridization complexes hybridize to the first and second capture oligonucleotides immobilized on the support surface.

3. The method of claim 1 or 2, wherein the sense binding length of the sense probe is shorter than the sense strand length of the sense strand by at least 1 nucleotide or the sense binding portion of the sense probe has a 5’ end that aligns with a 3’ end of the sense strand of the oligonucleotide duplex.

4. The method of any one of claims 1 to 3, wherein the antisense binding length of the antisense probe is shorter than the antisense strand length of the antisense strand by at least 1 nucleotide or the antisense binding portion of the antisense probe has a 5’ end that aligns with a 3’ end of the antisense strand of the oligonucleotide duplex.

5. The method of any one of claims 1 to 4, wherein the first oligonucleotide tag has a first oligonucleotide tag length and the first capture oligonucleotide has a first capture oligonucleotide length, wherein the first oligonucleotide tag length is the same as the first capture oligonucleotide length or shorter than the first capture oligonucleotide length.

6. The method of any one of claims 1 to 5, wherein the second oligonucleotide tag has a second oligonucleotide tag length and the second capture oligonucleotide has a second capture

oligonucleotide length, wherein the second oligonucleotide tag length is the same as the second capture oligonucleotide length or shorter than the second capture oligonucleotide length.

7. The method of claim 1, wherein (a)-(c) are performed concurrently or sequentially.

8. The method of any one of claims 1 to 7, wherein the hybridization conditions in (b) comprise:

(i) incubating the probes with the sample a first temperature to denature the sense and antisense strands of the oligonucleotide duplex; and

(ii) incubating the probes with the denatured sense and antisense strands of the oligonucleotide duplex at a second temperature to allow the sense and antisense probes to hybridize to the sense and antisense strands.

9. The method of claim 8, further comprising incubating the sense and antisense complexes at a hold temperature of about 2°C to about 8°C.

10. The method of claim 8, comprising a first temperature transition rate between steps (i) and (ii) of about 1 °C/s to about 2 °C/s.

11. The method according to any one of claims 1 to 10, wherein the sample comprises a plurality of oligonucleotide duplexes and the composition in (a) comprises a plurality of sets of probes, wherein each set of probes hybridizes with a unique sense or antisense strand of a unique oligonucleotide duplex.

12. The method of any one of claims 1 to 11, wherein (c) comprises incubating the support surface with the sense and antisense complexes for about 15 minutes to about 12 hours at a temperature of about 20 °C to about 40 °C.

13. The method of any one of claims 1 to 12, wherein the composition in (a) comprises about 20 pM to about 10 nM sense probe and/or about 20 pM to about 10 nM antisense probe.

14. The method of any one of claims 1 to 13, wherein the sample comprises a biological sample, an environmental sample, a manufacturing process sample, or combinations thereof.

15. The method of any one of claims 1 to 14, wherein the method has a limit of detection of less than about 200 pg/mL.

16. The method of any one of claims 1 to 15, wherein the support surface comprises one or more electrodes.

17. A composition comprising a set of probes, wherein the set of probes comprises:

(a) a sense probe comprising a first single stranded oligonucleotide tag that is complementary to at least a portion of a first capture oligonucleotide, a sense binding portion capable of hybridizing to a nucleotide sequence of a sense strand of an oligonucleotide duplex, and a first label; and

(b) an antisense probe comprising a second single stranded oligonucleotide tag that is complementary to at least a portion of a second capture oligonucleotide, an antisense binding portion capable of hybridizing to a nucleotide sequence of an antisense strand of the oligonucleotide duplex, and a second label,

wherein the sense binding portion of the sense probe has a sense binding length that is shorter than a sense strand length of the sense strand, and

wherein the antisense binding portion of the antisense strand has an antisense binding length that is shorter than an antisense strand length of the antisense strand.

18. A composition comprising:

(a) an oligonucleotide duplex comprising a sense strand and an antisense strand; and

(b) a set of probes comprising:

(i) a sense probe comprising a first single stranded oligonucleotide tag that is complementary to at least a portion of a first capture oligonucleotide, a sense binding portion capable of hybridizing to a nucleotide sequence of a sense strand of an oligonucleotide duplex, and a first label; and

(ii) an antisense probe comprising a second single stranded oligonucleotide tag that is complementary to at least a portion of a second capture oligonucleotide, an antisense binding portion capable of hybridizing to a nucleotide sequence of an antisense strand of the oligonucleotide duplex, and a second label,

wherein the sense binding portion of the sense probe has a sense binding length that is shorter than a sense strand length of the sense strand, and

wherein the antisense binding portion of the antisense strand has an antisense binding length that is shorter than an antisense strand length of the antisense strand.

19. A composition comprising one or more hybridization complexes, the hybridization complexes comprising:

(a) a sense complex comprising a sense probe hybridized to a sense strand of an oligonucleotide duplex, wherein the sense probe comprises a first single stranded oligonucleotide tag that is complementary to at least a portion of a first capture oligonucleotide, a sense binding portion capable of hybridizing to a nucleotide sequence of the sense strand of the oligonucleotide duplex, and a first label, wherein the sense binding portion of the sense probe has a sense binding length that is shorter than a sense strand length of the sense strand;

(b) an antisense complex comprising an antisense probe hybridized to an antisense strand of the oligonucleotide duplex, wherein the antisense probe comprises a second single stranded oligonucleotide tag that is complementary to at least a portion of a second capture oligonucleotide, an antisense binding portion capable of hybridizing to a nucleotide sequence of the antisense strand of the oligonucleotide duplex, and a second label, wherein the antisense binding portion of the antisense strand has an antisense binding length that is shorter than an antisense strand length of the antisense strand,

and combinations thereof.

20. A kit for carrying out the method of any one of claims 1 to 16.