

Multivalent Probes Having Single Nucleotide Resolution

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is claims priority to and the benefit of U.S. Provisional Application No. 62/213,812, filed September 3, 2015 and U.S. Provisional Application No. 62/292,690, filed February 8, 2016. Each of the above-mentioned applications is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] In nucleic acid detection, a trade-off exists between probes having high stability and probes having high specificity; for example, longer length probes have high melting temperatures and are highly stable, however, they lack specificity and are unable to detect single base substitutions. There exists a need for probes, compositions, methods, and kits for enabling accurate and robust enzyme- and amplification-free detection of DNA and RNA and with single base resolution.

SUMMARY OF THE INVENTION

[0003] The present invention relates to polymer strands, probes, compositions, methods, and kits for enabling accurate and robust enzyme- and amplification-free detection of DNA and RNA with single base resolution (*e.g.*, detection of a single nucleotide polymorphism (SNP), an insertion, and a deletion).

[0003a] In one aspect, the present invention provides a polymer strand pair comprising: a first polymer strand comprising at least (1) a first target binding region, (2) a first complementary region, and (3) a sequence-specific region a second polymer strand comprising at least (1) a second target binding region, and (2) a second complementary region; wherein the target of the first target binding region and the target of the second target binding region are in the same nucleic acid molecule and the target of the first target binding region is non-overlapping with the target of the second target binding region; and wherein the first complementary region is complementary to the second complementary region

[0003b] In another aspect, the present invention provides a composition comprising a plurality of polymer strand pairs disclosed herein, wherein a first polymer strand pair is capable of binding to a first nucleic acid molecule and an at least second polymer strand pair is capable of binding to an at least second nucleic acid molecule, wherein the first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0003c] In yet another aspect, the present invention provides a polymer strand trio comprising: (a) a polymer strand pair disclosed herein and (b) a capture polymer strand at least comprising: a region comprising at least one affinity moiety or comprising a region capable of binding to a single-stranded nucleic acid comprising at least one affinity moiety and a third target binding region capable of binding to the nucleic acid molecule, wherein the targets of the first, second, and third target binding regions are non-overlapping and in the same nucleic acid molecule.

[0003d] In yet another aspect, the present invention provides a composition comprising a plurality of polymer strand trios disclosed herein, wherein a first polymer strand trio is capable of binding to a first nucleic acid molecule and an at least second polymer strand trio is capable of binding to an at least second nucleic acid molecule, wherein the first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0003e] In yet another aspect, the present invention provides a composition comprising a plurality of partially double-stranded nucleic acid probes disclosed herein.

[0003f] In yet another aspect, the present invention provides a method for detecting a nucleic acid in a sample comprising: (1) contacting the sample with a polymer strand pair disclosed herein, wherein (a) the sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (b) the sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a

reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the nucleic acid molecule; wherein the at least one label monomer and the one or more label monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers, thereby detecting the nucleic acid molecule in the sample.

[0003g] In yet another aspect, the present invention provides a method for detecting a plurality of nucleic acids in a sample comprising: (1) contacting the sample with a first polymer strand pair and an at least second polymer strand pair disclosed herein, wherein (a) each sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either a first nucleic acid molecule or an at least second nucleic acid molecule; (b) each sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic acid molecule or the at least second nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic

acid molecule or the at least second nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the first nucleic acid molecule or the at least second nucleic acid molecule; wherein the at least one label monomer and the one or more label monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers for the first polymer strand pair and for the at least second polymer strand pair, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0003h] In yet another aspect, the present invention provides a method for detecting a nucleic acid in a sample comprising: (1) contacting the sample with the partially double-stranded nucleic acid probe disclosed herein, wherein (a) the sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (b) the sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the nucleic acid molecule; wherein the at least one label monomer and the one or more label monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another

monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers, thereby detecting the nucleic acid molecule in the sample.

[0003i] In yet another aspect, the present invention provides a method for detecting a plurality of nucleic acids in a sample comprising: (1) contacting the sample with a first partially double-stranded nucleic acid probe and an at least second partially double-stranded nucleic acid probe disclosed herein, wherein (a) each sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either a first nucleic acid molecule or an at least second nucleic acid molecule; (b) each sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic acid molecule or the at least second nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic acid molecule or the at least second nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the first nucleic acid molecule or the at least second nucleic acid molecule; wherein the at least one label monomer and the one or more label monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers for the first polymer strand pair and for

the at least second polymer strand pair, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0003j] In yet another aspect, the present invention provides a method for detecting a nucleic acid in a sample comprising: (1) contacting the sample with a polymer strand trio disclosed herein, wherein (a) the sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (b) the sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the nucleic acid molecule; wherein the at least one label monomer and the one or more label monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers, thereby detecting the nucleic acid molecule in the sample.

[0003k] In yet another aspect, the present invention provides a method for detecting a plurality of nucleic acids in a sample comprising: (1) contacting the sample with a first polymer strand trio and an at least second polymer strand trio disclosed herein, wherein (a) each sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one

complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either a first nucleic acid molecule or an at least second nucleic acid molecule; (b) each sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic acid molecule or the at least second nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic acid molecule or the at least second nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the first nucleic acid molecule or the at least second nucleic acid molecule; wherein the at least one label monomer and the one or more label monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers for the first polymer strand trio and for the at least second polymer strand trio, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0003l] In yet another aspect, the present invention provides a multivalent polymer strand comprising at least: a first target binding region, a second target binding region, a spacer between the first target binding region and the second target binding region; and a sequence-specific region; wherein the target of the first target binding region and the target of the second target binding region are in the same nucleic acid molecule and the target of the first target binding region is non-overlapping with the target of the second target binding region.

[0003m] In yet another aspect, the present invention provides a composition comprising a plurality of multivalent polymer strands disclosed herein, wherein a first multivalent polymer

strand is capable of binding to a first nucleic acid molecule and an at least second multivalent polymer strand is capable of binding to an at least second nucleic acid molecule, wherein the first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0003n] In yet another aspect, the present invention provides a multivalent polymer strand duo comprising: (a) a multivalent polymer strand disclosed herein, and (b) a capture polymer strand at least comprising: a region comprising at least one affinity moiety or comprising a region capable of binding to a single-stranded nucleic acid comprising at least one affinity moiety and a third target binding region capable of binding to the nucleic acid molecule, wherein the targets of the first, second, and third target binding regions are non-overlapping and in the same nucleic acid molecule.

[0003o] In yet another aspect, the present invention provides a composition comprising a plurality of multivalent polymer strand duos disclosed herein, wherein a first multivalent polymer strand duo is capable of binding to a first nucleic acid molecule and an at least second multivalent polymer strand duo is capable of binding to an at least second nucleic acid molecule, wherein the first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0003p] In yet another aspect, the present invention provides a method for detecting a nucleic acid in a sample comprising: (1) contacting the sample with a multivalent polymer strand disclosed herein, wherein (a) the sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (b) the sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded

oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the nucleic acid molecule; wherein the at least one label monomer and the one or more label monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers, thereby detecting the nucleic acid molecule in the sample.

[0003q] In yet another aspect, the present invention provides a method for detecting a plurality of nucleic acids in a sample comprising: (1) contacting the sample with a first multivalent polymer strand and an at least second multivalent polymer strand disclosed herein, wherein (a) each sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either a first nucleic acid molecule or an at least second nucleic acid molecule; (b) each sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic acid molecule or the at least second nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic acid molecule or the at least second nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the first nucleic acid molecule or the at least second nucleic acid molecule; wherein the at least one label monomer and the one or more label

monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers for the first multivalent polymer strand and for the at least second multivalent polymer strand, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0003r] In yet another aspect, the present invention provides a method for detecting a nucleic acid in a sample comprising: (1) contacting the sample with a multivalent polymer strand duo disclosed herein, wherein (a) the sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (b) the sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies the nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the nucleic acid molecule; wherein the at least one label monomer and the one or more label monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers, thereby detecting the nucleic acid molecule in the sample.

[0003s] In yet another aspect, the present invention provides a method for detecting a plurality of nucleic acids in a sample comprising: (1) contacting the sample with a first multivalent polymer strand duo and an at least second multivalent polymer strand duo disclosed herein, wherein (a) each sequence-specific region comprises at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either a first nucleic acid molecule or an at least second nucleic acid molecule; (b) each sequence-specific region is covalently attached to a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic acid molecule or the at least second nucleic acid molecule; (c) the sequence-specific region is bound or capable of being bound to a reporter probe, the reporter probe comprising at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone comprising at least two label attachment positions covalently linked in a linear combination, wherein each label attachment position is capable of binding at least one complementary single-stranded oligonucleotide comprising at least one label monomer, wherein a linear combination of labelled monomers identifies either the first nucleic acid molecule or the at least second nucleic acid molecule; or (d) the sequence-specific region comprises one or more label monomers, wherein the one or more label monomers identifies the first nucleic acid molecule or the at least second nucleic acid molecule; wherein the at least one label monomer and the one or more label monomers are selected from the group consisting of a fluorochrome, quantum dot, dye, enzyme, nanoparticle, mass tag, chemiluminescent marker, biotin, and another monomer that can be detected directly or indirectly; (2) detecting the linear combination of labelled monomers or the one or more label monomers for the first multivalent polymer strand duo and for the at least second multivalent polymer strand duo, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0003t] In yet another aspect, the present invention provides a kit comprising a composition disclosed herein and instructions for use.

[0004] A first example of the present invention relates to a polymer strand pair including a first polymer strand having at least (1) a first target binding region, (2) a first complementary region, and (3) a sequence-specific region and a second polymer strand including at least (1) a second target binding region and (2) a second complementary region. The target of the first target binding region and the target of the second target binding region are in the same nucleic acid molecule and the target of the first target binding region is non-overlapping with the target of the second target binding region. The first complementary region is complementary to the second complementary region.

[0005] A second example of the present disclosure relates to a method for detecting a nucleic acid in a sample including a step of contacting the sample with a polymer strand pair of the first example. This example includes a step of detecting a linear combination of labelled monomers or detecting one or more label monomers, thereby detecting the nucleic acid molecule in the sample.

[0006] In each example of the present disclosure that is directed to detecting a nucleic acid in a sample, each first polymer strand has a sequence-specific region that (a) includes at least two label attachment positions covalently linked in a linear combination, with each label attachment position capable of binding at least one complementary single-stranded oligonucleotide including at least one label monomer, and in which a linear combination of labelled monomers identifies the nucleic acid molecule; (b) is covalently attached to a single-stranded nucleic acid backbone, the backbone including at least two label attachment positions covalently linked in a linear combination, with each label attachment position capable of binding at least one complementary single-stranded oligonucleotide including at least one label monomer, and in which a linear combination of labelled monomers identifies the nucleic acid molecule; (c) is bound or capable of being bound to a reporter probe, the reporter probe including at least a binding portion complementary to the sequence-specific region and a single-stranded nucleic acid backbone, the backbone including at least two label attachment positions covalently linked in a linear combination, with each label attachment position capable of binding at least one complementary single-stranded oligonucleotide including at least one label monomer, in which a linear combination of labelled monomers identifies the nucleic acid molecule; or (d) includes one or more label monomers, with the one or more label monomers identifying the nucleic acid molecule. The one or more label monomer is selected from biotin, chemiluminescent marker, dye, enzyme, fluorochrome, nanoparticle, quantum dot, and another monomer that can be detected directly or indirectly.

[0007] A third example of the present disclosure relates to a composition including a plurality of polymer strand pairs of the first example. In this example, a first polymer strand pair is capable of binding to a first nucleic acid molecule and an at least second polymer strand pair is capable of binding to an at least second nucleic acid molecule. The first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0008] A fourth example of the present disclosure relates to a method for detecting a plurality of nucleic acids in a sample including a step of contacting the sample with a plurality of polymer strand pairs of the first example or of contacting the sample with a composition of the third example. This example includes a step of (1) detecting a linear combination of labelled monomers for a first polymer strand pair or detecting one or more label monomers on a first polymer strand pair and (2) detecting a linear combination of labelled monomers for an at least second polymer strand pair or detecting one more label monomers on an at least second polymer strand pair, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0009] A fifth example of the present disclosure relates to a polymer strand trio including a polymer strand pair of the first example and a capture polymer strand. The capture polymer strand includes at least (1) a region having at least one affinity moiety or a region capable of binding to a single-stranded nucleic acid including at least one affinity moiety and (2) a third target binding region capable of binding to the nucleic acid molecule. The targets of the first, second, and third target binding regions are non-overlapping and in the same nucleic acid molecule.

[0010] A sixth example of the present disclosure relates to a method for detecting a nucleic acid in a sample including a step of contacting the sample with a polymer strand trio of the fifth example. This example includes a step of detecting a linear combination of labelled monomers for the polymer strand trio or detecting one or more label monomers on the polymer strand trio, thereby detecting the nucleic acid molecule in the sample.

[0011] A seventh example of the present disclosure relates to a composition including a plurality of polymer strand trios of the fifth example. In this example, a first polymer strand trio is capable of binding to a first nucleic acid molecule and an at least second polymer strand trio is capable of binding to an at least second nucleic acid molecule. The first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0012] An eight example of the present disclosure relates to a method for detecting a plurality of nucleic acids in a sample including a step of contacting the sample with a plurality of polymer strand trios of the fifth example or of contacting the sample with a composition of the seventh example. This example includes a step of (1) detecting a linear combination of labelled monomers for a first polymer strand trio or detecting one or more label monomers on a first polymer strand trio and (2) detecting a linear combination of labelled monomers for an at least second polymer

strand trio or detecting one or more label monomers on an at least second polymer strand trio, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0013] A ninth example of the present disclosure relates to a partially double-stranded nucleic acid probe obtained when the first complementary region and the second complementary region of a polymer strand pair of the first example are hybridized. Upon hybridization, the first polymer strand and the second polymer strand form a partially-double stranded nucleic acid probe having each feature of the polymer strand pair of the first example.

[0014] A tenth example of the present disclosure relates to a method for detecting a nucleic acid in a sample including a step of contacting the sample with a partially double-stranded nucleic acid probe of the ninth example. This example includes a step of detecting a linear combination of labelled monomers for the partially double-stranded nucleic acid probe or detecting one or more label monomers on the partially double-stranded nucleic acid probe, thereby detecting the nucleic acid molecule in the sample.

[0015] An eleventh example of the present disclosure relates to a composition including a plurality of partially double-stranded nucleic acid probes of the ninth example. In this example, a first double-stranded nucleic acid probe is capable of binding to a first nucleic acid molecule and an at least second double-stranded nucleic acid probe is capable of binding to an at least second nucleic acid molecule. The first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0016] A twelfth example of the present disclosure relates to a method for detecting a plurality of nucleic acids in a sample including a step of contacting the sample with a plurality of partially double-stranded nucleic acid probes of the ninth example or of contacting the sample with a composition of the eleventh example. This example includes a step of (1) detecting a linear combination of labelled monomers for a first partially double-stranded nucleic acid probe or detecting one or more label monomers on a first partially double-stranded nucleic acid probe and (2) detecting a linear combination of labelled monomers for an at least second partially double-stranded nucleic acid probe or detecting one or more label monomers on an at least second partially double-stranded nucleic acid probe, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0017] A thirteenth example of the present disclosure relates to a composition including a plurality of partially double-stranded nucleic acid probes of the ninth example and a plurality of capture polymer strands. A first capture polymer strand at least includes (1) a region including at least one affinity moiety or a region capable of binding to a single-stranded nucleic acid including at least one affinity moiety and (2) a third target binding region that is capable of binding to a first nucleic acid molecule. Each at least second capture polymer strand at least includes (1) a region including at least one affinity moiety or a region capable of binding to a single-stranded nucleic acid comprising at least one affinity moiety and (2) a third target binding region that is capable of binding to an at least second nucleic acid molecule. The targets of each first, second, and third target binding regions are non-overlapping and in the same nucleic acid molecule. The first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0018] A fourteenth example of the present disclosure relates to a multivalent polymer strand including at least (1) a first target binding region, (2) a second target binding region, (3) a spacer between the first target binding region and the second target binding region, and (4) a sequence-specific region. The target of the first target binding region and the target of the second target binding region are in the same nucleic acid molecule and the target of the first target binding region is non-overlapping with the target of the second target binding region. The spacer may be polymer chain, *e.g.*, an oligonucleotide and polyethylene glycol.

[0019] A fifteenth example of the present disclosure relates to a method for detecting a nucleic acid in a sample including a step of contacting the sample with a multivalent polymer strand of the fourteenth example. This example includes a step of detecting a linear combination of labelled monomers or detecting one or more label monomers, thereby detecting the nucleic acid molecule in the sample.

[0020] A sixteenth example of the present disclosure relates to a composition including a plurality of multivalent polymer strands of the fourteenth example. In this example, a first multivalent polymer strand is capable of binding to a first nucleic acid molecule and an at least second multivalent polymer strand is capable of binding to an at least second nucleic acid molecule. The first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0021] A seventeenth example of the present disclosure relates to a method for detecting a plurality of nucleic acids in a sample including a step of contacting the sample with a plurality of multivalent polymer strands of the fourteenth example or of contacting the sample with a

composition of the sixteenth example. This example includes a step of (1) detecting a linear combination of labelled monomers for a first multivalent polymer strand or detecting one or more label monomers on a first multivalent polymer strand and (2) detecting a linear combination of labelled monomers for an at least second multivalent polymer strand or detecting one or more label monomers on an at least second multivalent polymer strand, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0022] An eighteenth example of the present disclosure relates to a multivalent polymer strand duo including a multivalent polymer strand of the fourteenth example and a capture polymer strand. The capture polymer strand includes at least (1) a region having at least one affinity moiety or a region capable of binding to a single-stranded nucleic acid including at least one affinity moiety and (2) a third target binding region capable of binding to the nucleic acid molecule. The targets of the first, second, and third target binding regions are non-overlapping and in the same nucleic acid molecule.

[0023] A nineteenth example of the present disclosure relates to a method for detecting a nucleic acid in a sample including a step of contacting the sample with a multivalent polymer strand duo of the eighteenth example. This example includes a step of detecting a linear combination of labelled monomers for the polymer strand trio or detecting one or more label monomers on the polymer strand trio thereby detecting the nucleic acid molecule in the sample.

[0024] A twentieth example of the present disclosure relates to a composition including a plurality of multivalent polymer strand duos of the eighteenth example. In this example, a first multivalent polymer strand duo is capable of binding to a first nucleic acid molecule and an at least second multivalent polymer strand duo is capable of binding to an at least second nucleic acid molecule. The first nucleic acid molecule differs from the at least second nucleic acid molecule.

[0025] A twenty-first example of the present disclosure relates to a method for detecting a plurality of nucleic acids in a sample including a step of contacting the sample with a plurality of multivalent polymer strand duos of the eighteenth example or of contacting the sample with a composition of the twentieth example. This example includes a step of (1) detecting a linear combination of labelled monomers for a first polymer strand trio or detecting one or more label monomers on a first polymer strand trio and (2) detecting a linear combination of labelled monomers for an at least second polymer strand trio or one or more label monomers on an at

least second polymer strand trio, thereby detecting the first nucleic acid molecule and the at least second nucleic acid molecule in the sample.

[0026] Any of the herein-described compositions may further comprise at least one probe capable of detecting a protein target.

[0027] Any of the herein-described methods may further comprise contacting a sample with at least one probe capable of detecting a protein target.

[0028] A twenty-second example of the present disclosure relates to a kit comprising a composition of the third example, of the seventh example, of the eleventh example, of the thirteenth example, of the sixteenth example, or of the twentieth example and instructions for use. Other components necessary to perform a method of any of the above examples may be included in a kit. The kit may further comprise at least one probe capable of detecting a protein target.

[0029] In each example of the present disclosure, a labeled oligonucleotide may be labeled with one or more detectable label monomers. The label may be at a terminus of an oligonucleotide, at a point within an oligonucleotide, or a combination thereof. Oligonucleotides may comprise nucleotides with amine-modifications, which allow coupling of a detectable label to the nucleotide. Labeled oligonucleotides of the present disclosure can be labeled with any of a variety of label monomers, such as a fluorochrome, quantum dot, dye, enzyme, nanoparticle, chemiluminescent marker, biotin, or other monomer known in the art that can be detected directly (*e.g.*, by light emission) or indirectly (*e.g.*, by binding of a fluorescently-labeled antibody). Preferred examples of a label that can be utilized by the disclosure are fluorophores. Several fluorophores can be used as label monomers for labeling nucleotides including, but not limited to GFP-related proteins, cyanine dyes, fluorescein, rhodamine, ALEXA Fluor™, Texas Red, FAM, JOE, TAMRA, and ROX. Several different fluorophores are known, and more continue to be produced, that span the entire spectrum.

[0030] In each example of the present disclosure, a label attachment position may be hybridized (non-covalently bound) with at least one labeled oligonucleotide. Alternately, a position may be hybridized with at least one oligonucleotide lacking a detectable label. Each position can hybridize to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 to 100 labeled (or unlabeled) oligonucleotides or more. The number of labeled oligonucleotides hybridized to each position depends on the length of the position and the size of the oligonucleotides. A position may be between about 12 to about 1500 nucleotides in length. The lengths of the

labeled (or unlabeled) oligonucleotides vary from about 12 to about 1500 nucleotides in length. In examples, the lengths of labeled (or unlabeled) oligonucleotides vary from about 800 to about 1300 ribonucleotides. In other examples, the lengths of labeled (or unlabeled) oligonucleotides vary from about 20 to about 55 deoxyribonucleotides; such oligonucleotides are designed to have melting/hybridization temperatures of between about 65 and about 85 °C, *e.g.*, about 80 °C. For example, a position of about 1100 nucleotides in length may hybridize to between about 25 and about 45 oligonucleotides comprising, each oligonucleotide about 45 to about 25 deoxyribonucleotides in length. In examples, each position is hybridized to about 34 labeled oligonucleotides of about 33 deoxyribonucleotides in length. The labeled oligonucleotides are preferably single-stranded DNA.

[0031] In each example of the present disclosure, labels associated with each position (*via* hybridization of a position with a labeled oligonucleotide) are spatially-separable and spectrally-resolvable from the labels of a preceding position or a subsequent position. An ordered series of spatially-separable and spectrally-resolvable labels of a probe is herein referred to as barcode or as a label code. The barcode or label code allows identification of a target nucleic acid or target protein that has been bound by a particular probe.

[0032] The terms “one or more”, “at least one”, and the like are understood to include but not be limited to at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000 or more and any number in between. Therefore, an “at least one complementary single-stranded oligonucleotide” may include, for example, 2 oligonucleotides, 6 oligonucleotides, and 10 oligonucleotides.

[0033] Conversely, the term “no more than” includes each value less than the stated value.

For example, “no more than 100 nucleotides” includes 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 79, 78, 77, 76, 75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38,

37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, and 0 nucleotides.

[0034] The terms “plurality”, “at least two”, “two or more”, “at least second”, and the like, are understood to include but not limited to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000 or more and any number in between.

Therefore, an “at least second polymer strand pair” includes, but is not limited to, 2 polymer strand pairs, 10 polymer strand pairs, 100 polymer strand pairs, and 1000 polymer strand pairs. Similarly, “at least second nucleic acid molecule” includes, but is not limited, to 2 nucleic acid molecules, 20 nucleic acid molecules, 40 nucleic acid molecules, and 60 nucleic acid molecules. Also, “at least second capture polymer strand “ includes, but is not limited, to 2 capture polymer strands, 500 capture polymer strands, 1000 capture polymer strands, and 5000 capture polymer strands. Moreover, “at least two label attachment positions” includes, but is not limited, 2 label attachment positions, 4 label attachment positions, 6 label attachment positions, and 8 label attachment positions.

[0035] In examples, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more nucleic acids, and any number there between, are detected. 800 or more different nucleic acids can be detected. In examples, detecting includes quantifying the abundance of each nucleic acid.

[0036] The probes and methods disclosed herein permit detection of somatic variants with about 5% allele frequency from as little as 5 ng fresh or formalin-fixed paraffin embedded (FFPE) genomic DNA (gDNA).

[0037] nCounter[®] probes, systems, and methods from NanoString Technologies[®], as described in US2003/0013091, US2007/0166708, US2010/0015607, US2010/0261026, US2010/0262374, US2010/0112710, US2010/0047924, US2014/0371088, US2014/0017688, and US2011/0086774), are a preferred means for identifying target proteins and/or target nucleic

acids. nCounter[®] probes, systems, and methods from NanoString Technologies[®] allow simultaneous multiplexed identification a plurality (800 or more) distinct target proteins and/or target nucleic acids. Each of the above-mentioned patent publications is incorporated herein by reference in its entirety. The above-mentioned nCounter[®] probes, systems, and methods from NanoString Technologies[®] can be combined with any example described herein.

[0038] A single nCounter[®] cartridge (*e.g.*, a single lane thereof) may be used for simultaneous multiplexed identification of a plurality distinct target proteins and/or target nucleic acids from the combination of the above-mentioned nCounter[®] probes, systems, and methods and the examples described herein.

[0039] Any example described herein can be combined with any other example as disclosed herein.

[0040] While the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure, which is defined by the scope of the appended claims. Other examples, advantages, and modifications are within the scope of the following claims.

[0041] The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0043] **Figure 1** shows exemplary polymer strands, polymer strand pairs, and partially double-stranded nucleic acid probes. **Figure 1A** shows a polymer strand pair comprising a first polymer strand including a first target binding region (in red) and a second target binding region (in green). **Figure 1B** shows a polymer strand pair having a first polymer strand comprising a label moiety (green circle) and a second polymer strand having an affinity moiety (asterisk). **Figure**