

CLAIMS

1. A method for identifying in a sample, one or more target ribonucleic acid molecules differing in sequence from other ribonucleic acid molecules in the sample due to alternative splicing, alternative transcript, alternative start site, alternative coding sequence, alternative non-coding sequence, exon insertion, exon deletion, intron insertion, translocation, mutation, or other rearrangement at the genome level, said method comprising:
 - providing a sample containing one or more target ribonucleic acid molecules potentially differing in sequence from other ribonucleic acid molecules;
 - contacting the sample with one or more enzymes capable of digesting dU containing nucleic acid molecules potentially present in the sample;
 - providing one or more oligonucleotide primers, each primer being complementary to the one or more target ribonucleic acid molecules;
 - blending the contacted sample, the one or more oligonucleotide primers, a deoxynucleotide mix including dUTP, and a reverse-transcriptase to form a reverse-transcription mixture;
 - generating complementary deoxyribonucleic acid (cDNA) molecules in the reverse transcription mixture, each cDNA molecule comprising a nucleotide sequence that is complementary to the target ribonucleic acid molecule and contains dU;
 - providing one or more oligonucleotide primer sets, each primer set comprising (a) a first oligonucleotide primer comprising a nucleotide sequence that is complementary to a portion of a cDNA nucleotide sequence adjacent to the target ribonucleic acid molecule sequence complement of the cDNA, and (b) a second oligonucleotide primer comprising a nucleotide sequence that is complementary to a portion of an extension product formed from the first oligonucleotide primer;
 - blending the reverse transcription mixture containing the cDNA molecules, the one or more oligonucleotide primer sets, a deoxynucleotide mix including dUTP, and a polymerase to form a polymerase reaction mixture;
 - subjecting the polymerase chain reaction mixture to one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment thereby forming one or more different primary extension products;
 - providing one or more oligonucleotide probe sets, each probe set comprising (a) a first oligonucleotide probe having a 5' primer-specific portion and a 3' target sequence-specific portion, and (b) a second oligonucleotide probe having a 5' target sequence-specific portion and a 3' primer-specific portion, wherein the first and second oligonucleotide probes of a probe set are configured to hybridize, in a base specific manner,

on complementary portions of a primary extension product corresponding to the target ribonucleic acid molecule sequence;

contacting the primary extension products with a ligase and the one or more oligonucleotide probe sets to form a ligation reaction mixture;

subjecting the ligation reaction mixture to one or more ligation reaction cycles whereby the first and second probes of the one or more oligonucleotide probe sets are ligated together to form ligated product sequences in the ligase reaction mixture, wherein each ligated product sequence comprises the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion;

providing one or more secondary oligonucleotide primer sets, each secondary oligonucleotide primer set comprising (a) a first secondary oligonucleotide primer comprising the same nucleotide sequence as the 5' primer-specific portion of the ligated product sequence and (b) a second secondary oligonucleotide primer comprising a nucleotide sequence that is complementary to the 3' primer-specific portion of the ligated product sequence;

blending the ligated product sequences, the one or more secondary oligonucleotide primer sets with one or more enzymes capable of digesting deoxyuracil (dU) containing nucleic acid molecules, a deoxynucleotide mix including dUTP, and a DNA polymerase to form a second polymerase chain reaction mixture;

subjecting the second polymerase chain reaction mixture to conditions suitable for digesting deoxyuracil (dU) containing nucleic acid molecules present in the second polymerase chain reaction mixture, and one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment thereby forming secondary extension products; and

detecting and distinguishing the secondary extension products in the sample thereby identifying the presence of one or more ribonucleic acid molecules differing in sequence from other ribonucleic acid molecules in the sample due to alternative splicing, alternative transcript, alternative start site, alternative coding sequence, alternative non-coding sequence, exon insertion, exon deletion, intron insertion, translocation, mutation, or other rearrangement at the genome level.

2. A method for identifying in a sample, one or more target ribonucleic acid molecules differing in sequence from other ribonucleic acid molecules in the sample due to alternative splicing, alternative transcript, alternative start site, alternative coding sequence, alternative non-coding sequence, exon insertion, exon deletion, intron insertion,

translocation, mutation, or other rearrangement at the genome level, said method comprising:

- providing a sample containing one or more target ribonucleic acid molecules potentially differing in sequence from other ribonucleic acid molecules;

- contacting the sample with one or more enzymes capable of digesting dU containing nucleic acid molecules potentially present in the sample;

- providing one or more oligonucleotide primers, each primer being complementary to the one or more target ribonucleic acid molecules;

- blending the contacted sample, the one or more oligonucleotide primers, a deoxynucleotide mix including dUTP, and a reverse-transcriptase to form a reverse-transcription mixture;

- generating complementary deoxyribonucleic acid (cDNA) molecules in the reverse transcription mixture, each cDNA molecule comprising a nucleotide sequence that is complementary to the target ribonucleic acid molecule and contains dU;

- providing one or more oligonucleotide primer sets, each primer set comprising (a) a first oligonucleotide primer comprising a nucleotide sequence that is complementary to a portion of a cDNA nucleotide sequence adjacent to the target ribonucleic acid molecule sequence complement of the cDNA, and (b) a second oligonucleotide primer comprising a nucleotide sequence that is complementary to a portion of an extension product formed from the first oligonucleotide primer;

- blending the reverse transcription mixture containing the cDNA molecules, the one or more oligonucleotide primer sets, a deoxynucleotide mix including dUTP, and a polymerase to form a polymerase reaction mixture;

- subjecting the polymerase chain reaction mixture to one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment thereby forming one or more different primary extension products;

- providing one or more oligonucleotide probe sets, each probe set comprising (a) a first oligonucleotide probe having a 5' portion and a 3' target nucleotide sequence-specific portion, and (b) a second oligonucleotide probe having a 5' target nucleotide sequence-specific portion and a 3' portion, wherein the 5' portion of the first oligonucleotide probe of the probe set is complementary to a portion of the 3' portion of the second oligonucleotide probe, wherein one probe of the probe set comprises a detectable signal generating moiety, and wherein the first and second oligonucleotide probes of a probe set are configured to hybridize, in a base specific manner, on complementary portions of a primary extension product corresponding to the target ribonucleic acid molecule sequence;

contacting the primary extension products with a ligase and the one or more oligonucleotide probe sets to form a ligation reaction mixture;

subjecting the ligation reaction mixture to one or more ligation reaction cycles whereby the first and second probes of the one or more oligonucleotide probe sets are ligated together to form ligated product sequences in the ligase reaction mixture, wherein each ligated product sequence comprises the 5' portion, the target-specific portions, the 3' portion, and the detectable signal generating moiety;

hybridizing the 5' portion of the ligated product sequence to its complementary 3' portion;

detecting signal from the detectable signal generating moiety that is produced upon said hybridizing; and

distinguishing the ligated product sequences in the sample based on said detecting to identify the presence of one or more ribonucleic acid molecules differing in sequence from other ribonucleic acid molecules in the sample due to alternative splicing, alternative transcript, alternative start site, alternative coding sequence, alternative non-coding sequence, exon insertion, exon deletion, intron insertion, translocation, mutation, or other rearrangement at the genome level.

3. A method for identifying, in a sample, one or more target micro-ribonucleic acid (miRNA) molecules differing in sequence from other miRNA molecules in the sample by one or more bases, said method comprising:

providing a sample containing one or more target miRNA molecules potentially differing in sequence from other miRNA molecules in the sample by one or more bases

contacting the sample with one or more enzymes capable of digesting dU containing nucleic acid molecules potentially present in the sample;

providing one or more oligonucleotide primer sets, each primer set comprising (a) a first oligonucleotide primer having a 5' stem-loop portion, a blocking group, an internal primer-specific portion within the loop region, and a 3' nucleotide sequence portion that is complementary to a 3' portion of the target miRNA molecule sequence, (b) a second oligonucleotide primer having a 3' nucleotide sequence portion that is complementary to a complement of the 5' end of the target miRNA molecule sequence, and a 5' primer-specific portion, (c) a third oligonucleotide primer comprising a nucleotide sequence that is the same as the internal primer-specific portion of the first oligonucleotide primer, and (d) a fourth oligonucleotide primer comprising a nucleotide sequence that is the same as the 5' primer-specific portion of the second oligonucleotide primer;

blending the contacted sample, the one or more first oligonucleotide primers of a primer set, a deoxynucleotide mix including dUTP, and a reverse transcriptase to form a reverse transcription reaction mixture wherein the first oligonucleotide primer hybridizes to the target miRNA molecule sequence, if present in the sample, and the reverse transcriptase extends the 3' end of the hybridized first oligonucleotide primer to generate an extended first oligonucleotide primer comprising the complement of the target miRNA molecule sequence;

blending the reverse transcription reaction mixture with the second, third, and fourth oligonucleotide primers of the primer set to form a polymerase reaction mixture under conditions effective for the one or more second oligonucleotide primers of a primer set to hybridize to the region of the extended first oligonucleotide primer comprising the complement of the target miRNA molecule sequence and extend to generate a primary extension product comprising the 5' primer-specific portion, a nucleotide sequence corresponding to the target miRNA molecule sequence, and the complement of the internal primer-specific portion;

subjecting the polymerase chain reaction mixture to one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment thereby forming a plurality of primary extension products;

blending the plurality of primary extension products with a ligase and one or more oligonucleotide probe sets to form a ligation reaction mixture, wherein each oligonucleotide probe set comprises (a) a first oligonucleotide probe having a 5' primer-specific portion and a 3' targetsequence-specific portion, and (b) a second oligonucleotide probe having a 5' target sequence-specific portion, a portion complementary to a primary extension product, and a 3' primer-specific portion, wherein the first and second oligonucleotide probes of a probe set are configured to hybridize, in a base specific manner, on complementary portions of a primary extension product corresponding to the target miRNA molecule sequence;

subjecting the ligation reaction mixture to one or more ligation reaction cycles whereby the first and second oligonucleotide probes of the one or more oligonucleotide probe sets are ligated together to form ligated product sequences in the ligation reaction mixture wherein each ligated product sequence comprises the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion;

providing one or more secondary oligonucleotide primer sets, each secondary oligonucleotide primer set comprising (a) a first secondary oligonucleotide primer comprising the same nucleotide sequence as the 5' primer-specific portion of the ligated product sequence and (b) a second secondary oligonucleotide primer comprising a

nucleotide sequence that is complementary to the 3' primer-specific portion of the ligated product sequence;

blending the ligated product sequences, the one or more secondary oligonucleotide primer sets, with one or more enzymes capable of digesting deoxyuracil (dU) containing nucleic acid molecules, a deoxynucleotide mix including dUTP, and a DNA polymerase to form a second polymerase chain reaction mixture;

subjecting the second polymerase chain reaction mixture to conditions suitable for digesting deoxyuracil (dU) containing nucleic acid molecules present in the second polymerase chain reaction mixture, and one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment thereby forming secondary extension products; and

detecting and distinguishing the secondary extension products in the sample thereby identifying one or more target miRNA molecules differing in sequence from other miRNA molecules in the sample by one or more bases.

4. A method for identifying, in a sample, one or more target micro-ribonucleic acid (miRNA) molecules differing in sequence from other miRNA molecules in the sample by one or more bases, said method comprising:

providing a sample containing one or more target miRNA molecules potentially differing in sequence from other miRNA molecules in the sample by one or more bases

contacting the sample with one or more enzymes capable of digesting dU containing nucleic acid molecules potentially present in the sample;

providing one or more oligonucleotide primer sets, each primer set comprising (a) a first oligonucleotide primer having a 5' stem-loop portion, a blocking group, an internal primer-specific portion within the loop region, and a 3' nucleotide sequence portion that is complementary to a 3' portion of the target miRNA molecule sequence, (b) a second oligonucleotide primer having a 3' nucleotide sequence portion that is complementary to a complement of the 5' end of the target miRNA molecule sequence, and a 5' primer-specific portion, (c) a third oligonucleotide primer comprising a nucleotide sequence that is the same as the internal primer-specific portion of the first oligonucleotide primer, and (d) a fourth oligonucleotide primer comprising a nucleotide sequence that is the same as the 5' primer-specific portion of the second oligonucleotide primer;

blending the contacted sample, the one or more first oligonucleotide primers of a primer set, a deoxynucleotide mix including dUTP, and a reverse transcriptase to form a reverse transcription reaction mixture wherein the first oligonucleotide primer hybridizes to

the target miRNA molecule sequence, if present in the sample, and the reverse transcriptase extends the 3' end of the hybridized first oligonucleotide primer to generate an extended first oligonucleotide primer comprising the complement of the target miRNA molecule sequence;

blending the reverse transcription reaction mixture with the second, third, and fourth oligonucleotide primers of the primer set to form a polymerase reaction mixture under conditions effective for the one or more second oligonucleotide primers of a primer set to hybridize to the region of the extended first oligonucleotide primer comprising the complement of the target miRNA molecule sequence and extend to generate a primary extension product comprising the 5' primer-specific portion, a nucleotide sequence corresponding to the target miRNA molecule sequence, and the complement of the internal primer-specific portion;

subjecting the polymerase chain reaction mixture to one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment thereby forming a plurality of primary extension products;

blending the plurality of primary extension products with a ligase and one or more oligonucleotide probe sets to form a ligation reaction mixture, wherein each oligonucleotide probe set comprises (a) a first oligonucleotide probe having a 5' portion and a 3' target nucleotide sequence-specific portion, and (b) a second oligonucleotide probe having a 5' target nucleotide sequence-specific portion and a 3' portion, wherein the 5' portion of the first oligonucleotide probe of the probe set is complementary to a portion of the 3' portion of the second oligonucleotide probe, wherein one probe of the probe set comprises a detectable signal generating moiety, and wherein the first and second oligonucleotide probes of a probe set are configured to hybridize, in a base specific manner, on complementary portions of a primary extension product corresponding to the target miRNA molecule sequence;

subjecting the ligation reaction mixture to one or more ligation reaction cycles whereby the first and second oligonucleotide probes of the one or more oligonucleotide probe sets are ligated together to form ligated product sequences in the ligation reaction mixture wherein each ligated product sequence comprises the 5' portion, the target-specific portions, the 3' portion, and the detectable signal generating moiety;

hybridizing the 5' portion of the ligated product sequence to its complementary 3' portion;

detecting signal from the detectable signal generating moiety that is produced upon said hybridizing; and

distinguishing ligated product sequences in the sample based on said detecting to identify the presence one or more target miRNA molecules differing in sequence from other miRNA molecules in the sample by one or more bases.

5. A method for identifying, in a sample, one or more target micro-ribonucleic acid (miRNA) molecules differing in sequence from other miRNA molecules in the sample by one or more bases, said method comprising:

providing a sample containing one or more miRNA molecules potentially differing in sequence from other miRNA molecules by one or more base differences;

contacting the sample with one or more enzymes capable of digesting dU containing nucleic acid molecules potentially present in the sample

blending the contacted sample with a ligase and a first oligonucleotide probe comprising a 5' phosphate, a 5' stem-loop portion, an internal primer-specific portion within the loop region, a blocking group, and a 3' nucleotide sequence that is complementary to a 3' portion of the target miRNA molecule sequence to form a ligation reaction;

ligating the target miRNA molecule sequence at its 3' end to the 5' phosphate of the first oligonucleotide probe to generate a chimeric nucleic acid molecule comprising the target miRNA molecule sequence, if present in the sample, appended to the first oligonucleotide probe;

providing one or more oligonucleotide primer sets, each primer set comprising (a) a first oligonucleotide primer comprising a 3' nucleotide sequence that is complementary to a complement of the 5' end of the target miRNA molecule sequence, and a 5' primer-specific portion, (b) a second oligonucleotide primer comprising a nucleotide sequence that is complementary to the internal primer-specific portion of the first oligonucleotide probe, and (c) a third oligonucleotide primer comprising a nucleotide sequence that is the same as the 5' primer-specific portion of the first oligonucleotide primer;

blending the chimeric nucleic acid molecule, the one or more second oligonucleotide primers, a deoxynucleotide mix including dUTP, and a reverse transcriptase to form a reverse transcription reaction mixture, wherein the one or more second oligonucleotide primers of a primer set hybridizes to the internal primer specific portion of the chimeric nucleic acid molecule and extends at its 3' end to generate a complement of the chimeric nucleic acid molecule, if present in the sample;

blending the reverse transcription reaction mixture with the first and third oligonucleotide primers of a primer set to form a polymerase reaction mixture;

subjecting the polymerase chain reaction mixture to one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and

an extension treatment thereby forming primary extension products comprising the 5' primer-specific portion, a nucleotide sequence corresponding to the target miRNA molecule sequence, and the complement of the internal primer-specific portion.

blending the primary extension products with a ligase and one or more oligonucleotide probe sets to form a ligation reaction mixture, wherein each oligonucleotide probe set comprises (a) a first oligonucleotide probe having a 5' primer-specific portion and a 3' target sequence-specific portion, and (b) a second oligonucleotide probe having a 5' target sequence-specific portion, a portion complementary to a primary extension product, and a 3' primer-specific portion, wherein the first and second oligonucleotide probes of a probe set are configured to hybridize, in a base specific manner, on complementary portions of a primary extension product corresponding to the target miRNA molecule sequence;

subjecting the ligation reaction mixture to one or more ligation reaction cycles whereby the first and second oligonucleotide probes of the one or more oligonucleotide probe sets are ligated together to form ligated product sequences in the ligation reaction mixture, wherein each ligated product sequence comprises the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion;

providing one or more secondary oligonucleotide primer sets, each secondary oligonucleotide primer set comprising (a) a first secondary oligonucleotide primer comprising the same nucleotide sequence as the 5' primer-specific portion of the ligated product sequence and (b) a second secondary oligonucleotide primer comprising a nucleotide sequence that is complementary to the 3' primer-specific portion of the ligated product sequence;

blending the ligated product sequences and the one or more secondary oligonucleotide primer sets, with one or more enzymes capable of digesting deoxyuracil (dU) containing nucleic acid molecules, a deoxynucleotide mix including dUTP, and a DNA polymerase to form a second polymerase chain reaction mixture;

subjecting the second polymerase chain reaction mixture to conditions suitable for digesting deoxyuracil (dU) containing nucleic acid molecules present in the second polymerase chain reaction mixture, and one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment thereby forming secondary extension products; and

detecting and distinguishing the secondary extension products in the sample thereby identifying one or more target miRNA molecules differing in sequence from other miRNA molecules in the sample by one or more bases.

6. A method for identifying, in a sample, one or more target micro-ribonucleic acid (miRNA) molecules differing in sequence from other miRNA molecules in the sample by one or more bases, said method comprising:

providing a sample containing one or more miRNA molecules potentially differing in sequence from other miRNA molecules by one or more base differences;

contacting the sample with one or more enzymes capable of digesting dU containing nucleic acid molecules potentially present in the sample

blending the contacted sample with a ligase and a first oligonucleotide probe comprising a 5' phosphate, a 5' stem-loop portion, an internal primer-specific portion within the loop region, a blocking group, and a 3' nucleotide sequence that is complementary to a 3' portion of the target miRNA molecule sequence to form a ligation reaction;

ligating the target miRNA molecule sequence at its 3' end to the 5' phosphate of the first oligonucleotide probe to generate a chimeric nucleic acid molecule comprising the target miRNA molecule sequence, if present in the sample, appended to the first oligonucleotide probe;

providing one or more oligonucleotide primer sets, each primer set comprising (a) a first oligonucleotide primer comprising a 3' nucleotide sequence that is complementary to a complement of the 5' end of the target miRNA molecule sequence, and a 5' primer-specific portion, (b) a second oligonucleotide primer comprising a nucleotide sequence that is complementary to the internal primer-specific portion of the first oligonucleotide probe, and (c) a third oligonucleotide primer comprising a nucleotide sequence that is the same as the 5' primer-specific portion of the first oligonucleotide primer;

blending the chimeric nucleic acid molecule, the one or more second oligonucleotide primers, a deoxynucleotide mix including dUTP, and a reverse transcriptase to form a reverse transcription reaction mixture, wherein the one or more second oligonucleotide primers of a primer set hybridizes to the internal primer specific portion of the chimeric nucleic acid molecule and extends at its 3' end to generate a complement of the chimeric nucleic acid molecule, if present in the sample;

blending the reverse transcription reaction mixture with the first and third oligonucleotide primers of a primer set to form a polymerase reaction mixture;

subjecting the polymerase chain reaction mixture to one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment thereby forming primary extension products comprising the 5' primer-specific portion, a nucleotide sequence corresponding to the target miRNA molecule sequence, and the complement of the internal primer-specific portion;

blending the primary extension products with a ligase and one or more oligonucleotide probe sets to form a ligation reaction mixture, wherein each oligonucleotide probe set comprises (a) a first oligonucleotide probe having a 5' portion and a 3' target nucleotide sequence-specific portion, and (b) a second oligonucleotide probe having a 5' target nucleotide sequence-specific portion and a 3' portion, wherein the 5' portion of the first oligonucleotide probe of the probe set is complementary to a portion of the 3' portion of the second oligonucleotide probe, wherein one probe of the probe set comprises a detectable signal generating moiety, and wherein the first and second oligonucleotide probes of a probe set are configured to hybridize, in a base specific manner, on complementary portions of a primary extension product corresponding to the target miRNA molecule sequence;

subjecting the ligation reaction mixture to one or more ligation reaction cycles whereby the first and second oligonucleotide probes of the one or more oligonucleotide probe sets are ligated together to form ligated product sequences in the ligation reaction mixture wherein each ligated product sequence comprises the 5' portion, the target-specific portions, the 3' portion, and the detectable signal generating moiety;

hybridizing the 5' portion of the ligated product sequence to its complementary 3' portion;

detecting signal from the detectable signal generating moiety that is produced upon said hybridizing; and

distinguishing ligated product sequences in the sample based on said detecting to identify the presence one or more target miRNA molecules differing in sequence from other miRNA molecules in the sample by one or more bases.

7. The method of any one of claims 1, 3, or 5, wherein the second oligonucleotide probe of the oligonucleotide probe set further comprises a unitaq detection portion, thereby forming ligated product sequences comprising the 5' primer-specific portion, the target-specific portions, the unitaq detection portion, and the 3' primer-specific portion, said method further comprising:

providing one or more unitaq detection probes, wherein each unitaq detection probe hybridizes to a complementary unitaq detection portion and said detection probe comprises a quencher molecule and a detectable label that are separated from each other;

adding the one or more unitaq detection probes to the second polymerase chain reaction mixture; and

hybridizing the one or more unitaq detection probes to complementary unitaq detection portions on the ligated product sequence or complement thereof during said

subjecting the second polymerase chain reaction mixture to conditions suitable for one or more polymerase chain reaction cycles, whereby the quencher molecule and the detectable label are cleaved from the one or more unitaq detection probes during the extension treatment, whereby said detecting involves the detection of the cleaved detectable label.

8. The method of any one of claims 1, 3, or 5, said method further comprising:

providing one or more oligonucleotide detection probes, wherein each oligonucleotide detection probe hybridizes to a ligation product junction portion or its complement, and said detection probe comprises a quencher molecule and a detectable label that are separated from each other;

adding the one or more oligonucleotide detection probes to the second polymerase chain reaction mixture; and

hybridizing the one or more oligonucleotide detection probes to complementary detection portions on the ligated product sequence or complement thereof during said subjecting the second polymerase chain reaction mixture to conditions suitable for one or more polymerase chain reaction cycles, whereby the quencher molecule and the detectable label are cleaved from the one or more oligonucleotide detection probes during said extension treatment, whereby said detecting involves the detection of the cleaved detectable label.

9. The method of any one of claims 2, 4, or 6, said method further comprising:

immobilizing at least one strand of each primary extension product on a solid support prior to or subsequent to said ligating, wherein said ligated product sequence is hybridized to the immobilized primary extension product; and

removing unligated oligonucleotides probes and non-target specific ligation products from the sample after said ligating; and

denaturing the ligation product sequence from the immobilized primary extension product prior to said hybridizing.

10. The method of any one of claims 1-9, wherein the 3' portion of the first oligonucleotide probe of the oligonucleotide probe set comprises a cleavable nucleotide or nucleotide analogue and a blocking group, such that the 3' end is unsuitable for polymerase extension or ligation, said method further comprising;

cleaving the cleavable nucleotide or nucleotide analog of the first oligonucleotide probe when said probe is hybridized to its complementary target nucleotide sequence of the primary extension product, thereby liberating a 3'OH on the first oligonucleotide probe prior to said ligating.