

WHAT IS CLAIMED IS:

1. A method for identifying, in a sample, one or more nucleic acid molecules containing a target nucleotide sequence differing from nucleotide sequences in other nucleic acid molecules in the sample, or other samples, by one or more nucleotides, one or more copy numbers, one or more transcript sequences, and/or one or more methylated residues, said method comprising:
 - providing a sample containing one or more nucleic acid molecules potentially containing the target nucleotide sequence differing from the nucleotide sequences in other nucleic acid molecules by one or more nucleotides, one or more copy numbers, one or more transcript sequences, and/or one or more methylated residues;
 - providing one or more enzymes capable of digesting deoxyuracil (dU) containing nucleic acid molecules present in the sample;
 - providing one or more primary oligonucleotide primer sets, each primary oligonucleotide primer set comprising (a) a first primary oligonucleotide primer that comprises a nucleotide sequence that is complementary to a sequence adjacent to the target nucleotide sequence and (b) a second primary oligonucleotide primer that comprises a nucleotide sequence that is complementary to a portion of an extension product formed from the first primary oligonucleotide primer;
 - blending the sample, the one or more primary oligonucleotide primer sets, the one or more enzymes capable of digesting deoxyuracil (dU) containing nucleic acid molecules in the sample, a deoxynucleotide mix including dUTP, and a DNA polymerase to form a polymerase chain reaction mixture;
 - subjecting the polymerase chain reaction mixture to conditions suitable for digesting deoxyuracil (dU) containing nucleic acid molecules present in the 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 primary extension products comprising the target nucleotide sequence or a complement thereof;
 - 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 nucleotide sequence-specific portion, and (b) a second oligonucleotide probe having a 5' target nucleotide sequence-specific portion and a 3' primer-specific portion, and wherein the first

and second oligonucleotide probes of a probe set are configured to hybridize, in a base specific manner, on a complementary target nucleotide sequence of a primary extension product;

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, the 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 to identify the presence of one or more nucleic acid molecules containing target nucleotide sequences differing from nucleotide sequences in other nucleic acid molecules in the sample by one or more nucleotides, one or more copy numbers, one or more transcript sequences, and/or one or more methylated residues.

2. A method for identifying, in a sample, one or more nucleic acid molecules containing a target nucleotide sequence differing from nucleotide sequences in other nucleic acid molecules in the sample, or other samples, by one or more nucleotides, one or more copy numbers, one or more transcript sequences, and/or one or more methylated residues, said method comprising:

providing a sample containing one or more nucleic acid molecules potentially containing the target nucleotide sequence differing from the nucleotide sequences in other nucleic acid molecules by one or more nucleotides, one or more copy numbers, one or more transcript sequences, and/or one or more methylated residues;

5 providing one or more enzymes capable of digesting deoxyuracil (dU) containing nucleic acid molecules present in the sample;

providing one or more primary oligonucleotide primer sets, each primary oligonucleotide primer set comprising (a) a first primary oligonucleotide primer that comprises a nucleotide sequence that is complementary to a sequence adjacent to the target nucleotide
10 sequence and (b) a second primary oligonucleotide primer that comprises a nucleotide sequence that is complementary to a portion of an extension product formed from the first primary oligonucleotide primer;

blending the sample, the one or more primary oligonucleotide primer sets, the one or more enzymes capable of digesting deoxyuracil (dU) containing nucleic acid molecules in the
15 sample, a deoxynucleotide mix including dUTP, and a DNA polymerase to form a polymerase chain reaction mixture;

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

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
25 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
30 configured to hybridize, in a base specific manner, on a complementary target nucleotide sequence of a primary extension product;

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;

5 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 of one or more nucleic acid molecules containing target nucleotide sequences differing from nucleotide sequences in other nucleic acid molecules in the sample by one or more nucleotides, one or more copy numbers, one or more transcript sequences, and/or one or more methylated residues.

3. The method of claim 1 or 2 further comprising:
15 contacting the sample with at least a first methylation sensitive enzyme to form a restriction enzyme reaction mixture prior to, or concurrent with, said blending to form a polymerase chain reaction mixture, wherein said first methylation sensitive enzyme cleaves nucleic acid molecules in the sample that contain one or more unmethylated residues within at least one methylation sensitive enzyme recognition sequence, and whereby said detecting
20 involves detection of one or more nucleic acid molecules containing the target nucleotide sequence, wherein said nucleic acid molecules originally contained one or more methylated residues.

4. The method of claim 3, wherein the first primary oligonucleotide primer
25 comprises a nucleotide sequence that is complementary to a region of the target nucleotide sequence that is upstream of the one or more methylated residues and the second primary oligonucleotide primer comprises a nucleotide sequence that is the same as a region of the target nucleotide sequence that is downstream of the one or more methylated residues.

5. The method of claim 3 further comprising:
30 subjecting the restriction enzyme reaction mixture to a bisulfite treatment under conditions suitable to convert unmethylated cytosine residues to uracil residues prior to said blending to form a polymerase chain reaction mixture, wherein the first primary oligonucleotide

primer of the provided primary oligonucleotide primer set comprises a nucleotide sequence that is complementary to the bisulfite-treated target nucleotide sequence containing the one or more methylated, uncleaved restriction sites and the second primary oligonucleotide primer of the provided primary oligonucleotide primer set comprises a nucleotide sequence that is
5 complementary to a portion of the extension product formed from the first oligonucleotide primer.

6. The method of claim 5 further comprising:
providing one or more second methylation sensitive enzymes that cleave nucleic
10 acid molecules containing unmethylated residues within a methylation sensitive enzyme recognition sequence; and
blending the at least one second methylation sensitive enzyme with the polymerase chain reaction mixture comprising the bisulfite-treated restriction enzyme reaction mixture to form a second restriction enzyme reaction mixture, wherein said second methylation
15 sensitive enzyme cleaves nucleic acid molecules potentially present in the sample that contain one or more unmethylated residues within at least one methylation sensitive enzyme recognition sequence during said hybridization treatment.

7. The method of claim 5, wherein one or both primary oligonucleotide
20 primers of the primary oligonucleotide primer set have a 3' portion comprising a cleavable nucleotide or nucleotide analogue and a blocking group, such that the 3' end of said primer or primers is unsuitable for polymerase extension, said method further comprising:
cleaving the cleavable nucleotide or nucleotide analog of one or both
oligonucleotide primers during said hybridization treatment, thereby liberating free 3'OH ends
25 on one or both oligonucleotide primers prior to said extension treatment.

8. The method of claim 7, wherein the first primary oligonucleotide primer
of the primary oligonucleotide primer set comprises a 5' portion having a nucleotide sequence
that is the same as a nucleotide sequence portion in a bisulfite-treated unmethylated target
30 sequence to which the primary oligonucleotide primer hybridizes to, but has one or more nucleotide sequence mismatches to a corresponding nucleotide sequence portion in the bisulfite-treated methylated target sequence.

9. The method of claim 8, wherein the DNA polymerase lacks 5' nuclease activity, 3' nuclease activity, and strand displacing activity.

10. The method of claim 4 further comprising;
5 providing one or more blocking oligonucleotides capable of hybridizing to a region of the bisulfite-treated target nucleotide sequence containing unmethylated residues; and contacting the polymerase chain reaction mixture comprising the bisulfite-treated restriction enzyme reaction mixture with said one or more blocking oligonucleotides prior to said
10 subjecting, wherein said one or more blocking oligonucleotides hybridize to complementary bisulfite treated target nucleic acid sequences during said hybridization treatment and impede primary oligonucleotide primer extension during said extension treatment.

11. The method of claim 1 or 2, wherein one or both primary oligonucleotide primers of the primary oligonucleotide primer set have a 3' portion comprising a cleavable
15 nucleotide or nucleotide analogue and a blocking group, such that the 3' end of said primer or primers is unsuitable for polymerase extension, said method further comprising:
cleaving the cleavable nucleotide or nucleotide analog of one or both oligonucleotide primers during said hybridization treatment, thereby liberating free 3'OH ends on one or both oligonucleotide primers prior to said extension treatment.

20 12. The method of claim 10, wherein the cleavable nucleotide comprises one or more RNA bases.

25 13. The method of claim 12, wherein the first primary oligonucleotide primer of the primary oligonucleotide primer set comprises a 5' portion having a nucleotide sequence that is the same as a nucleotide sequence portion in a wildtype nucleic acid molecule to which the primary oligonucleotide primer hybridizes to, but has one or more nucleotide sequence mismatches to a corresponding nucleotide sequence portion in the target nucleic acid molecule.

30 14. The method of claim 13, wherein the one or more sequence mismatches is located two nucleotide bases from the 5' end of the first primary oligonucleotide primer, and/or three nucleotide bases from the 5' end of the first primary oligonucleotide primer.

15. The method of claim 13, wherein the DNA polymerase lacks 5' nuclease activity, 3' nuclease activity, and strand displacing activity.

5 16. 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:

10 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;

15 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

20 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

25 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;

30 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.

17. 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:
- 5 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
 - 10 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
 - 15 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)
 - 20 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;
 - 25 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
 - 30 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.

18. 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;

5 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
10 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;

15 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

20 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.

19. A method for identifying, in a sample, one or more target micro-
25 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

30 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.

20. 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;

5 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

10 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.

21. A method for identifying, in a sample, one or more target micro-
15 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
20 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;

25 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
30 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

5 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.

22. The method of any one of claims 1, 16, 18, or 20, 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:

15 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;

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

20 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.

23. The method of any one of claims 1, 16, 18, or 20, said method further comprising:

25 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;

30 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.

5

24. The method of any one of claims 2, 17, 19, or 21, 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

10 hybridized to the immobilized primary extension product; and

removing unligated oligonucleotide 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.

15

25. The method of any one of claims 1-24, 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;

20

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.

25

26. The method of any one of claims 1-25, wherein the second oligonucleotide probe has, at its 5' end, an overlapping identical nucleotide with the 3' end of the first oligonucleotide probe, and, upon hybridization of the first and second oligonucleotide probes of a probe set at adjacent positions on a complementary target nucleotide sequence of a primary extension product to form a junction, the overlapping identical nucleotide of the second oligonucleotide probe forms a flap at the junction with the first oligonucleotide probe, said

30

method further comprising:

cleaving the overlapping identical nucleotide of the second oligonucleotide probe with an enzyme having 5' nuclease activity thereby liberating a phosphate at the 5' end of the second oligonucleotide probe prior to said ligating.

5 27. The method of any one of claims 1-26, wherein the one or more oligonucleotide probe sets further comprise a third oligonucleotide probe having a target-specific portion, wherein the second and third oligonucleotide probes of a probe set are configured to hybridize adjacent to one another on the target nucleotide sequence with a junction between them to allow ligation between the second and third oligonucleotide probes to form a ligated product
10 sequence comprising the first, second, and third oligonucleotide probes of a probe set.

 28. A method for identifying, in a sample, one or more nucleic acid molecules containing a target nucleotide sequence differing from nucleotide sequences in other nucleic acid molecules in the sample, or other samples, by one more methylated residues, said method
15 comprising:

 providing a sample containing one or more nucleic acid molecules potentially comprising the target nucleotide sequence differing from the nucleotide sequences in other nucleic acid molecules by one or more methylated residues;

 contacting the sample with one or more enzymes capable of digesting deoxyuracil
20 (dU) containing nucleic acid molecules present in the sample;

 contacting the sample with one or more methylation sensitive enzymes to form a restriction enzyme reaction mixture, wherein the one or more said methylation sensitive enzyme cleaves nucleic acid molecules in the sample that contain one or more unmethylated residues within at least one methylation sensitive enzyme recognition sequence;

25 providing one or more primary oligonucleotide primer sets, each primary oligonucleotide primer set comprising (a) first primary oligonucleotide primer comprises a nucleotide sequence that is complementary to a region of the target nucleotide sequence that is upstream of the one or more methylated residues and (b) a second primary oligonucleotide primer comprises a nucleotide sequence that is the same as a region of the target nucleotide
30 sequence that is downstream of the one or more methylated residues;

 blending the restriction enzyme reaction mixture with the one or more primary oligonucleotide primer sets, a deoxynucleotide mix including dUTP, and a DNA polymerase to form a primary polymerase chain reaction mixture;

subjecting the primary 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 target nucleotide sequence or a complement thereof;

5 providing one or more secondary oligonucleotide primer sets, each secondary oligonucleotide primer set comprising first and second nested oligonucleotide primers capable of hybridizing to the primary extension products;

blending the primary extension products, the one or more secondary oligonucleotide primer sets, a deoxynucleotide mix including dUTP, and a DNA polymerase to
10 form a secondary polymerase chain reaction mixture;

subjecting the second 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 secondary extension products; and

detecting and distinguishing the secondary extension products in the sample to
15 identify the presence of one or more nucleic acid molecules containing target nucleotide sequences differing from nucleotide sequences in other nucleic acid molecules in the sample by one or more methylated residues.

29. The method of claim 28, said method further comprising:

20 providing one or more oligonucleotide detection probes, wherein each oligonucleotide detection probe hybridizes to a target nucleotide sequence in the primary extension product 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
25 chain reaction mixture; and

hybridizing the one or more oligonucleotide detection probes to complementary portions of the primary extension products during said subjecting, whereby the quencher molecule and the detectable label are cleaved from the one or more oligonucleotide detection probes during said extension treatment of the one or more polymerase chain reaction cycles of
30 the secondary polymerase chain reaction mixture, whereby said detecting involves the detection of the cleaved detectable label.

30. The method of claim 28, wherein one or both primary oligonucleotide primers of the primary oligonucleotide primer set have a 3' portion comprising a cleavable nucleotide or nucleotide analogue and a blocking group, such that the 3' end of said primer or primers is unsuitable for polymerase extension, said method further comprising:

5 cleaving the cleavable nucleotide or nucleotide analog of one or both primary oligonucleotide primers during the hybridization treatment of the one or more polymerase chain reaction cycles of the primary polymerase chain reaction mixture, thereby liberating free 3'OH ends on one or both primary oligonucleotide primers prior to said extension treatment.

10 31. The method of claim 28, wherein the primary oligonucleotide primers of the primary oligonucleotide primer set comprise an identical or substantially identical 5' nucleotide sequence portion that is between about 6 to 20 bases in length.

15 32. The method of any one of claims 1-31, wherein the sample is selected from the group consisting of tissue, cells, serum, blood, plasma, amniotic fluid, sputum, urine, bodily fluids, bodily secretions, bodily excretions, cell-free circulating nucleic acids, cell-free circulating tumor nucleic acids, cell-free circulating fetal nucleic acids in pregnant woman, circulating tumor cells, tumor, tumor biopsy, and exosomes.

20 33. The method of any one of claims 1-31, wherein the one or more target nucleotide sequences are low-abundance nucleic acid molecules comprising one or more nucleotide base mutations, insertions, deletions, translocations, splice variants, miRNA variants, alternative transcripts, alternative start sites, alternative coding sequences, alternative non-coding sequences, alternative splicings, exon insertions, exon deletions, intron insertions, or other
25 rearrangement at the genome level and/or methylated nucleotide bases.

30 34. The method of claim 33, wherein the low-abundance nucleic acid molecules with one or more nucleotide base mutations, insertions, deletions, translocations, splice variants, miRNA variants, alternative transcripts, alternative start sites, alternative coding sequences, alternative non-coding sequences, alternative splicings, exon insertions, exon deletions, intron insertions, or other rearrangement at the genome level, and/or methylated nucleotide bases are identified and distinguished from a high-abundance of nucleic acid molecules in the sample having a similar nucleotide sequence as the low abundance nucleic acid

5 molecules but without the one or more nucleotide base mutations, insertions, deletions, translocations, splice variants, miRNA variants, alternative transcripts, alternative start sites, alternative coding sequences, alternative non-coding sequences, alternative splicings, exon insertions, exon deletions, intron insertions, or other rearrangement at the genome level, and/or methylated nucleotide bases.

10 35. The method of claim 34, wherein the copy number of one or more low-abundance target nucleotide sequences are quantified relative to the copy number of the high-abundance nucleic acid molecules in the sample.

36. The method of any one of claims 1-31, wherein the one or more target nucleotide sequences are quantified or enumerated.

15 37. The method of claim 36, wherein the one or more target nucleotide sequences are quantified or enumerated relative to other nucleotide sequences in the sample or other samples undergoing the identical subsequent steps.

20 38. The method of claim 37, wherein the relative copy number of one or more target nucleotide sequences are quantified or enumerated.

39. The method of any one of claims 1-31 further comprising: diagnosing or prognosing a disease state based on said identifying.

25 40. The method of any one of claims 1-31 further comprising: distinguishing a genotype or disease predisposition based on said identifying.

30 41. A device for use in combination with a microtiter plate to simultaneously add liquids to two or more wells in a row and/or column of the microtiter plate, said microtiter plate having opposed top and bottom surfaces with the top surface having openings leading into the wells and the bottom surface defining closed ends of the wells, said device comprising:
a first layer defined by first and second boundaries with metering chambers extending between the first and second boundaries of said first layer and in fluid communication with one another, said first layer being configured to be fitted, in an operative position,

proximate to said microtiter plate with the first boundary of said first layer being closest to the top surface of said microtiter plate and each of the metering chambers being in fluid communication with an individual well in a row and/or column of the microtiter plate, said first layer further comprising a filling chamber in fluid communication with one or more of said metering chambers and

a second layer defined by first and second boundaries with a filling port extending between the first and second boundaries of said second layer, said second layer being configured to be fitted, in an operative position, proximate to said first layer with the first boundary of said second layer closest to the second boundary of said first layer and the filling port being aligned with the filling chamber, wherein, when said first layer, said second layer, and said microtiter plate are positioned with respect to one another in their operative positions, liquid entering said device through the filling port will pass through the filling chamber, the metering chambers, and into two or more wells in a row and/or column of said microtiter plate.

42. The device of claim 41, wherein said second layer comprises air passages extending between the first and second boundaries of said second layer with the air passages being in alignment with the metering chambers.

43. The device of claim 41 further comprising:
an intermediate layer defined by first and second boundaries with intermediate layer passages extending between the first and second boundaries of said intermediate layer, said intermediate layer being configured to be fitted, in an operative position, between said microtiter plate and said first layer with the first boundary of said intermediate layer adjacent to the top surface of said microtiter plate and the second boundary of said intermediate layer adjacent to the first boundary of the first layer and one of the intermediate layer passages being aligned with an individual well in a row and/or column of the microtiter plate, wherein, when said first layer, said second layer, said intermediate layer, and said microtiter plate are positioned with respect to one another in their operative positions, liquid entering said device through the filling port will pass through the filling chamber, the metering chambers, the intermediate layer passages, and into the wells of said microtiter plate.

44. The device of claim 43, wherein the intermediate layer passages comprise hydrophobic walls.

45. The device of claim 43, wherein the intermediate layer passages are burst valves.

5 46. The device of claim 41, wherein said first layer further comprises an overflow chamber that is in fluid communication with the filling chamber.

47. The device of claim 46, wherein said intermediate layer further comprises an overflow passage connecting the overflow chamber with said filling chamber.

10 48. The device of claim 46, wherein said second layer has an overflow air passage extending between the first and second boundaries of said second layer with the overflow air passage being in alignment with the overflow chamber.

15 49. The device of claim 43 further comprising:
a third layer having first and second boundaries with a filling port connector extending between the first and second boundaries of said third layer, said third layer being configured to be fitted, in an operative position, between said first and second layers with the first boundary of said third layer adjacent to the second boundary of said first layer and the
20 filling port connector being aligned with the filling port, wherein, when said first layer, said second layer, said third layer, said intermediate layer, and said microtiter plate are positioned with respect to one another in their operative positions, liquid entering said device through the filling port will pass through the filling port connector, the filling chamber, the metering chambers, the intermediate layer passages, and into the wells of said microtiter plate.

25 50. The device of claim 41, wherein the device is configured to permit the metering chambers to be filled by capillary action.

30 51. The device of claim 41, wherein the device is configured to permit the metering chambers to be filled by mechanical force.

52. The device of claim 41, wherein said first layer is provided with a pair of spaced filling chambers on opposite ends of the metering chambers and said second layer is

provided with a pair of spaced filling ports, each in fluid communication with one of the pair of spaced filling chambers, whereby one of the pair of spaced filling ports provides liquid to one of the pair of spaced filling chambers and half of the metering chambers, while the other one of the pair of spaced filling ports provides liquid to the other of the pair of filling chambers and the other half of the metering chambers.

53. The device of claim 41, wherein said first layer is provided with a pair of spaced filling chambers on opposite ends of the metering chambers and said second layer being provided with a pair of spaced filling ports, each in fluid communication with one of the pair of spaced filling chambers, whereby one of the pairs of spaced filling ports provides liquid to one of the pair of filling chambers and all of the metering chambers, while the other one of the pair of spaced filling ports provides liquid to the other of the pair of filling chambers and all of the metering chambers.

54. The device of claim 41, wherein the device is configured to fill two or more rows and columns of said microtiter plate with liquid.

55. The device of claim 41, wherein said first layer has a first region having the metering chambers in two or more rows, and a second region having the metering chambers in two or more columns with the first and second regions being displaced from each other.

56. The device of any one of claims 41-55, wherein said layers are integral giving said device a monolithic structure.

57. A method of adding liquids to two or more wells in a row and/or column of a microtiter plate having opposed top and bottom surfaces with the top surface having openings leading into the wells and the bottom surface defining closed ends of the wells, said method comprising:

providing a device comprising:

a first layer having first and second boundaries with metering chambers extending between the first and second boundaries of said first layer and in fluid communication with one another, said first layer being configured to be fitted, in an operative position, proximate to said microtiter plate with the first boundaries of said first

layer being closest to the top surface of said microtiter plate and one of the metering chambers being in fluid communication with an individual well in a row and/or column of the microtiter plate, said first layer further comprising a filling chamber in fluid communication with one or more of said metering chambers and

5 a second layer having first and second boundaries with a filling port extending between the first and second boundaries of said second layer, said second layer being configured to be fitted, in an operative position, proximate to said first layer with the first boundary of said second layer being closest to the second boundary of said first layer and the filling port being aligned with the filling chamber, wherein, when said first
10 layer, said second layer, and said microtiter plate are positioned with respect to one another in their operative positions, liquid entering said device through the filling port will pass through the filling chamber, the metering chambers, and into two or more wells in a row and/or column of said microtiter plate;

 filling the device with liquid; and

15 discharging liquid in the device into two or more wells in a row and/or column of said microtiter plate.

58. The method of claim 57, wherein the device further comprises:

 an intermediate layer having first and second boundaries with intermediate layer
20 passages extending between the first and second boundaries of said intermediate layer, said intermediate layer being configured to be fitted, in an operative position, between said microtiter plate and the said first layer with the first boundary of said intermediate layer adjacent to the top surface of said microtiter plate and the second boundary of said intermediate layer adjacent to the first boundary of the first layer and one of the intermediate layer passages being aligned with an
25 individual well in a row and/or column of the microtiter plate, wherein, when said first layer, said second layer, said intermediate layer, and said microtiter plate are positioned with respect to one another in their operative positions, liquid entering said device through the filling port will pass through the filling chamber, the metering chambers, the intermediate layer passages, and into the wells of said microtiter plate.

30

59. The method of claim 58, wherein the intermediate layer passages comprise hydrophobic walls.

60. The method of claim 58, wherein the intermediate layer passages are burst valves.

61. The method of claim 58, wherein the device further comprises:
a third layer having first and second boundaries with a filling port connector
5 extending between the first and second boundaries of said third layer, said third layer being
configured to be fitted, in an operative position, between said first and second layers with the
first boundary of said third layer adjacent to the second boundary of said second layer and the
filling port connector being aligned with the filling port, wherein, when said first layer, said
second layer, said third layer, said intermediate layer, and said microtiter plate are positioned
10 with respect to one another in their operative positions, liquid entering said device through the
filling port will pass through the filling port connector, the filling chamber, the metering
chambers, the intermediate layer passages, and into the wells of said microtiter plate.

62. The method of claim 57, wherein said filling is carried out by capillary
15 action.

63. The method of claim 57, wherein said discharging is carried out by
mechanical force.

64. The method of claim 57, wherein said first layer is provided with a pair of
20 spaced filling chambers on opposite sides of the metering chambers and said second layer is
provided with a pair of spaced filling ports, each in fluid communication with one of the pair of
spaced filling chambers, whereby, during said filling, one of the pair of spaced filling ports
provides liquid to one of the pair of spaced filling chambers and half of the metering chambers,
25 while the other one of the pair of spaced filling ports provides liquid to the other of the pair of
spaced filling chambers and the other half of the metering chambers.

65. The method of claim 57, wherein said first layer being provided with a
pair of spaced filling chambers on opposite ends of the metering chambers and said second layer
30 being provided with a pair of spaced filling ports, each in fluid communication with one of the
pair of spaced filling chambers, whereby, during said filling, one of the pairs of spaced filling
ports provides liquid to one of the pair of spaced filling chambers and all of the metering

chambers, while the other one of the pair of spaced filling port provides liquid to the other of the pair of spaced filling chambers and all of the metering chambers.

5 66. The method of claim 57, wherein the device is configured to fill two or more rows and columns of said microtiter plate with liquid, during said filling.

 67. The method of claim 57, wherein said first layer has a first region having the metering chambers in two or more rows, and a second region having the metering chambers in two or more columns with the first and second regions being displaced from each other.

10 68. The method of any one of claims 57-67, wherein said layers are integral giving said device a monolithic structure.

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.