**CLAIMS**

1. A method for identifying, in a sample, one or more parent nucleic acid molecules containing a target nucleotide sequence differing from nucleotide sequences of other parent 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, said method comprising:

providing a sample containing one or more parent nucleic acid molecules potentially containing the target nucleotide sequence differing from the nucleotide sequences of other parent 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;

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 in the parent nucleic acid molecule 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 first primary oligonucleotide primers of the primary oligonucleotide primer sets, the one or more enzymes capable of digesting deoxyuracil (dU)-containing nucleic acid molecules, a deoxynucleotide mix, and a DNA polymerase to form one or more polymerase extension reaction mixtures;

subjecting the one or more polymerase extension reaction mixtures to conditions suitable for digesting deoxyuracil (dU)-containing nucleic acid molecules present in the polymerase extension reaction mixtures and for carrying out one or more polymerase extension reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment, thereby forming primary extension products comprising nucleotide sequences complementary to the target nucleotide sequence;

blending the one or more polymerase extension reaction mixtures comprising the primary extension products, the one or more second primary oligonucleotide primers of the primary oligonucleotide primer sets, the one or more enzymes capable of digesting deoxyuracil (dU)-containing nucleic acid molecules in the reaction mixtures, a deoxynucleotide mix including dUTP, and a DNA polymerase to form one or more first polymerase chain reaction mixtures;

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

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 a complementary target nucleotide sequence of a secondary extension product;

blending the one or more first polymerase chain reaction products with a ligase, and the one or more oligonucleotide probe sets to form one or more ligation reaction mixtures;

subjecting the one or more ligation reaction mixtures 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, when hybridized to their complementary sequence, to form ligated product sequences in the ligation reaction mixtures 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 one or more second polymerase chain reaction mixtures;

subjecting the one or more second polymerase chain reaction mixtures to conditions suitable for digesting deoxyuracil (dU)-containing nucleic acid molecules present in the second polymerase chain reaction mixtures and for carrying out one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an

extension treatment thereby forming one or more second polymerase chain reaction products; and

detecting and distinguishing the one or more second polymerase chain reaction products in the one or more second polymerase chain reaction mixtures to identify the presence of one or more parent nucleic acid molecules containing target nucleotide sequences differing from nucleotide sequences in other parent 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 parent nucleic acid molecules containing a target nucleotide sequence differing from nucleotide sequences in other parent nucleic acid molecules in the sample by one or more methylated residues, said method comprising:

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

subjecting the nucleic acid molecules in the sample to a bisulfite treatment under conditions suitable to convert unmethylated cytosine residues to uracil residues;

providing one or more enzymes capable of digesting deoxyuracil (dU)-containing nucleic acid molecules;

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 in the bisulfite-treated parent nucleic acid molecules adjacent to the bisulfite-treated target nucleotide sequence containing the one or more methylated residue 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 bisulfite-treated sample, the one or more first primary oligonucleotide primers of the one or more primary oligonucleotide primer sets, a deoxynucleotide mix, and a DNA polymerase to form one or more polymerase extension reaction mixtures;

subjecting the one or more polymerase extension reaction mixtures to conditions suitable for one or more polymerase extension reaction cycles comprising a denaturation

treatment, a hybridization treatment, and an extension treatment, thereby forming primary extension products comprising the complement of the bisulfite-treated target nucleotide sequence;

blending the one or more polymerase extension reaction mixtures comprising the primary extension products, the one or more secondary primary oligonucleotide primers of the one or more primary 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 one or more first polymerase chain reaction mixtures;

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

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’ bisulfite-treated target nucleotide sequence-specific or complement sequence-specific portion, and (b) a second oligonucleotide probe having a 5’ bisulfite-treated target nucleotide sequence-specific or complement 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 nucleotide sequence of a first polymerase chain reaction product;

blending the first polymerase chain reaction products with a ligase and the one or more oligonucleotide probe sets to form one or more ligation reaction mixtures;

subjecting the one or more ligation reaction mixtures 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, when hybridized to complementary sequences, to form ligated product sequences in the ligation reaction mixture wherein each ligated product sequence comprises the 5’ primer-specific portion, the bisulfite-treated target nucleotide sequence-specific or complement sequence-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 one or more second polymerase chain reaction mixtures;

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

detecting and distinguishing the second polymerase chain reaction products in the one or more second polymerase chain reaction mixtures to identify the presence of one or more nucleic acid molecules containing target nucleotide sequences differing from nucleotide sequences in other parent nucleic acid molecules in the sample by one or more methylated residues.

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

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

subjecting the nucleic acid molecules in the sample to a bisulfite treatment under conditions suitable to convert unmethylated cytosine residues to uracil 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 in the bisulfite-treated parent nucleic acid molecules adjacent to the bisulfite-treated target nucleotide sequence containing the one or more methylated residue 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 bisulfite-treated sample, the one or more first primary oligonucleotide primers of the one or more primary oligonucleotide primer sets, a deoxynucleotide mix, and a DNA polymerase to form one or more polymerase extension reaction mixtures;

subjecting the one or more polymerase extension reaction mixtures to conditions suitable for one or more polymerase extension reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment, thereby forming primary extension products comprising the complement of the bisulfite treated target nucleotide sequence;

blending the one or more polymerase extension reaction mixtures comprising the primary extension products, the one or more secondary primary oligonucleotide primers of the one or more primary oligonucleotide primer sets, the one or more enzymes capable of digesting deoxyuracil (dU)-containing nucleic acid molecules in the reaction mixture, a deoxynucleotide mix, and a DNA polymerase to form one or more first polymerase chain reaction mixtures;

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

providing one or more secondary oligonucleotide primer sets, each secondary oligonucleotide primer set comprising (a) a first secondary oligonucleotide primer having a 3’ portion that is complementary to a portion of a first polymerase chain reaction product formed from the first primary oligonucleotide primer and (b) a second secondary oligonucleotide primer having a 3’ portion that comprises a nucleotide sequence that is complementary to a portion of a first polymerase chain reaction product formed from the first secondary oligonucleotide primer;

blending the first polymerase chain reaction products, 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 one or more second polymerase chain reaction mixtures;

subjecting the one or more second polymerase chain reaction mixtures to conditions suitable for digesting deoxyuracil (dU)-containing nucleic acid molecules present in the second polymerase chain reaction mixtures and for carrying out two or more polymerase

chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment thereby forming second polymerase chain reaction products; and

detecting and distinguishing the second polymerase chain reactions products in the one or more second polymerase chain reaction mixtures to identify the presence of one or more parent nucleic acid molecules containing target nucleotide sequences differing from nucleotide sequences in other parent nucleic acid molecules in the sample by one or more methylated residues.

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

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

subjecting the nucleic acid molecules in the sample to a bisulfite treatment under conditions suitable to convert unmethylated cytosine residues to uracil 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 having a 5’ primer-specific portion and a 3’ portion that comprises a nucleotide sequence that is complementary to a sequence in the bisulfite-treated parent nucleic acid molecules adjacent to the bisulfite-treated target nucleotide sequence containing the one or more methylated residue and (b) a second primary oligonucleotide primer having a 5’ primer-specific portion and a 3’ portion that comprises a nucleotide sequence that is complementary to a portion of an extension product formed from the first primary oligonucleotide primer;

blending the bisulfite treated sample, the one or more first primary oligonucleotide primers of the one or more primary oligonucleotide primer sets, a deoxynucleotide mix, and a DNA polymerase to form one or more polymerase extension reaction mixtures;

subjecting the one or more polymerase extension reaction mixtures to conditions suitable for one or more polymerase extension reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment, thereby forming primary

extension products comprising the complement of the bisulfite treated target nucleotide sequence;

blending the one or more polymerase extension reaction mixtures comprising the primary extension products, the one or more secondary primary oligonucleotide primers of the one or more primary oligonucleotide primer sets, the one or more enzymes capable of digesting deoxyuracil (dU)-containing nucleic acid molecules in the reaction mixture, a deoxynucleotide mix, and a DNA polymerase to form one or more first polymerase chain reaction mixtures;

subjecting the one or more first polymerase chain reaction mixtures to conditions suitable for digesting deoxyuracil (dU)-containing nucleic acid molecules present in the polymerase chain reaction mixtures and for carrying out one or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment, thereby forming first polymerase chain reactions products comprising the bisulfite-treated target nucleotide sequence or a complement thereof;

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 first polymerase chain reaction products or their complements and (b) a second secondary oligonucleotide primer comprising a nucleotide sequence that is complementary to the 3’ primer-specific portion of the first polymerase chain reaction products or their complements;

blending the primary polymerase chain reaction 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 one or more second polymerase chain reaction mixtures;

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

detecting and distinguishing the second polymerase chain reactions products in the one or more second polymerase chain reaction mixtures to identify the presence of one or more parent nucleic acid molecules containing target nucleotide sequences differing from nucleotide sequences in other parent nucleic acid molecules in the sample by one or more methylated residues.

5. The method of any one of claims 1 through 4 further comprising:

contacting the sample with DNA repair enzymes to repair damaged DNA, abasic sites, oxidized bases, or nicks in the DNA.

6. The method of any one of claims 2 and 5 further comprising:

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 one or more polymerase extension reaction mixtures, 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 involves detection of one or more parent nucleic acid molecules containing the target nucleotide sequence, wherein said parent nucleic acid molecules originally contained one or more methylated residues.

7. The method of any one of claims 2 and 5 further comprising:

contacting the sample with an immobilized methylated nucleic acid binding protein or antibody to selectively bind and enrich for methylated nucleic acid in the sample.

8. The method of any one of claims 1 through 4, wherein primers from said one or more primary or secondary oligonucleotide primer sets comprise a portion that has no or one nucleotide sequence mismatch when hybridized in a base-specific manner to the target nucleic acid sequence or bisulfite-converted methylated nucleic acid sequence or complement sequence thereof, but have one or more additional nucleotide sequence mismatches that interferes with polymerase extension when primers from said one or more primary or secondary oligonucleotide primer sets hybridize in a base-specific manner to a corresponding nucleotide sequence portion in wildtype nucleic acid sequence or bisulfite-converted unmethylated nucleic acid sequence or complement sequence thereof.

9. The method of any one of claims 1 through 4, wherein one or both primary oligonucleotide primers of the primary oligonucleotide primer set and/or one or both secondary oligonucleotide primers of the secondary 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 on one or both oligonucleotide primers prior to said extension treatment.

10. The method of claim 9, wherein primers from said one or more primary or secondary oligonucleotide primer sets comprise a sequence that differs from the target nucleic acid sequence or bisulfite-converted methylated nucleic acid sequence or complement sequence thereof, said difference is located two or three nucleotide bases from the liberated free 3'OH end.

11. The method of claim 9, wherein the cleavable nucleotide comprises one or more RNA bases.

12. The method of any one of claims 1 through 4 further comprising;

providing one or more blocking oligonucleotide primers comprising one or more mismatched bases at the 3' end or one or more nucleotide analogs and a blocking group at the 3' end, such that the 3' end of said blocking oligonucleotide primer is unsuitable for polymerase extension when hybridized in a base-specific manner to wildtype nucleic acid sequence or bisulfite-converted unmethylated nucleic acid sequence or complement sequence thereof, wherein said blocking oligonucleotide primer comprises a portion having a nucleotide sequence that is the same as a nucleotide sequence portion in the wildtype nucleic acid sequence or bisulfite-converted unmethylated nucleic acid sequence or complement sequence thereof to which the blocking oligonucleotide primer hybridizes but has one or more nucleotide sequence mismatches to a corresponding nucleotide sequence portion in the target nucleic acid sequence or bisulfite-converted methylated nucleic acid sequence or complement sequence thereof and

blending the one or more blocking oligonucleotide primers with the sample or subsequent products prior to a polymerase extension reaction, polymerase chain reaction, or ligation reaction, whereby during hybridization said one or more blocking oligonucleotide primers preferentially hybridize in a base-specific manner to a wildtype nucleic acid sequence or bisulfite-converted unmethylated nucleic acid sequence or complement sequence thereof, thereby interfering with polymerase extension or ligation during reaction of a primer or probes hybridized in a base-specific manner to the wildtype sequence or bisulfite-converted unmethylated sequence or complement sequence thereof.

13. The method of claim 3, wherein the first secondary oligonucleotide primer has a 5’ primer-specific portion and the second secondary oligonucleotide primer has a 5’ primer-specific portion, said one or more secondary oligonucleotide primer sets further comprising a third secondary oligonucleotide primer comprising the same nucleotide sequence as the 5’ primer-specific portion of the first secondary oligonucleotide primer and (d) a fourth secondary oligonucleotide primer comprising the same nucleotide sequence as the 5’ primer-specific portion of the second secondary oligonucleotide primer.

14. 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;

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

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 ATP and a Poly(A) polymerase to form a Poly(A) polymerase reaction mixture;

subjecting the Poly(A) polymerase reaction mixture to conditions suitable for appending homopolymer A to the 3’ ends of the one or more target miRNA molecules potentially present in the sample;

providing one or more primary oligonucleotide primer sets, each primer set comprising (a) a first primary oligonucleotide primer comprising a 5’ primer-specific portion, an internal poly dT portion, and a 3’ portion comprising from 1 to 10 bases complementary to the 3’ end of the target miRNA, wherein the first primary oligonucleotide primer may be the same or may differ from other first primary oligonucleotide primers in other sets, and (b) a second primary oligonucleotide primer comprising a 5’ primer-specific portion and a 3’ portion, wherein the second primary oligonucleotide primer may be the same or may differ from other second primary oligonucleotide primers in other sets;

blending the Poly(A) polymerase reaction mixture, 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 reverse transcriptase and a DNA polymerase or a DNA polymerase with reverse-

transcriptase activity to form one or more reverse-transcription/polymerase chain reaction mixtures;

subjecting the one or more reverse-transcription/polymerase chain reaction mixtures to conditions suitable for digesting deoxyuracil (dU)-containing nucleic acid molecules present in the reverse-transcription/polymerase chain reaction mixtures, then to conditions suitable for generating complementary deoxyribonucleic acid (cDNA) molecules to the target miRNA sequences with 3' polyA tails, and 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 reverse-transcription/polymerase chain reaction products comprising the 5' primer-specific portion of the second primary oligonucleotide primer, a nucleotide sequence corresponding to the target miRNA molecule sequence, a poly dA region, and the complement of the 5' primer-specific portion of the first primary oligonucleotide primer, and complements thereof;

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, a portion complementary to the one or more reverse-transcription/polymerase chain reaction products, 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, to complementary portions of the one or more reverse-transcription/polymerase chain reaction products corresponding to the target miRNA molecule sequence, or complement thereof;

contacting the one or more reverse-transcription/polymerase chain reaction products with a ligase and the one or more oligonucleotide probe sets to form one or more ligation reaction mixtures;

subjecting the one or more ligation reaction mixtures to one or more ligation reaction cycles whereby the first and second oligonucleotide probes of the one or more oligonucleotide probe sets, when hybridized to their complement, 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 one or more first polymerase chain reaction mixtures;

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

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

15. 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;

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

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 ATP and a Poly(A) polymerase to form a Poly(A) polymerase reaction mixture;

subjecting the Poly(A) polymerase reaction mixture to conditions suitable for appending a homopolymer A to the 3’ ends of the one or more target miRNA molecules potentially present in the sample;

providing one or more primary oligonucleotide primer sets, each primer set comprising (a) a first primary oligonucleotide primer comprising a 5’ primer-specific portion, an internal poly dT portion, and a 3’ portion comprising from 1 to 10 bases complementary to the 3’ end of the target miRNA, wherein the first primary oligonucleotide primer may be the same or may differ from other first primary oligonucleotide primers in other sets, and (b) a second

primary oligonucleotide primer comprising a 5’ primer-specific portion and a 3’ portion, wherein the second primary oligonucleotide primer may be the same or may differ from other second primary oligonucleotide primers in other sets;

blending the Poly(A) polymerase reaction mixture potentially comprising target miRNA sequences with 3’ polyA tails, the one or more primary oligonucleotide primer sets, a deoxynucleotide mix, and a reverse transcriptase and a DNA polymerase or a DNA polymerase with reverse-transcriptase activity to form one or more reverse-transcription/polymerase chain reaction mixtures;

subjecting the one or more reverse-transcription/polymerase chain reaction mixtures to conditions suitable for generating complementary deoxyribonucleic acid (cDNA) molecules to the target miRNA sequences with 3’ polyA tails, and 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 reverse-transcription/polymerase chain reaction products comprising the 5’ primer-specific portion of the second primary oligonucleotide primer, a nucleotide sequence corresponding to the target miRNA molecule sequence, a poly dA region, and the complement of the 5’ primer-specific portion of the first primary oligonucleotide primer, and complements thereof;

providing one or more secondary oligonucleotide primer sets, each secondary oligonucleotide primer set comprising (a) a first secondary oligonucleotide primer having a 5’ primer-specific portion and a 3’ portion that is complementary to a portion of a reverse-transcription/polymerase chain reaction product formed from the first primary oligonucleotide primer and (b) a second secondary oligonucleotide primer having a 5’ primer-specific portion and a 3’ portion that comprises a nucleotide sequence that is complementary to a portion of a reverse-transcription/polymerase chain reaction product formed from the first secondary oligonucleotide primer;

blending the reverse-transcription/polymerase chain reaction products, the one or more secondary oligonucleotide primer sets, a deoxynucleotide mix, and a DNA polymerase to form one or more first polymerase chain reaction mixtures;

subjecting the one or more first polymerase chain reaction mixtures to conditions suitable for two or more polymerase chain reaction cycles comprising a denaturation treatment, a hybridization treatment, and an extension treatment, thereby forming first polymerase chain reaction products comprising a 5’ primer-specific portion, a nucleotide sequence corresponding to the target miRNA molecule sequence or a complement thereof, and a complement of the other 5’ primer-specific portion;

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

blending the first polymerase chain reaction products, the one or more tertiary 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 one or more second polymerase chain reaction mixtures;

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

detecting and distinguishing the second polymerase chain reaction products in the one or more reactions thereby identifying one or more target miRNA molecules differing in sequence from other miRNA molecules in the sample by one or more bases.

16. The method of claim 14 or claim 15 wherein the 3’ portion of the second primary oligonucleotide primer comprises ribo-G and/or G nucleotide analogue, wherein the reverse transcriptase appends two or three cytosine nucleotides to the 3’ end of the complementary deoxyribonucleic acid products of the target miRNAs, enabling transient hybridization to the 3’ end of the second primary oligonucleotide primer, enabling the reverse transcriptase to undergo strand switching and to extend the complementary deoxyribonucleic acid products to include the complementary sequence of the 5’ primer-specific portion of the second primary oligonucleotide primer to form the one or more different first polymerase chain reaction products comprising a 5’ primer-specific portion, a nucleotide sequence portion corresponding to the target miRNA molecule sequence or a complement thereof, a further portion, and a complement of the other 5’ primer-specific portion.

17. The method of claim 14 or claim 15 wherein the 3’ portion of the second primary oligonucleotide primers contains from 6 to 14 bases comprising, from 5’ to 3’, three ribo-G or G bases, followed by additional bases that are the same as the 5’ end of the target

miRNA sequences, wherein the reverse transcriptase appends two or three cytosine residues to the 3’ end of the initial complementary deoxyribonucleic acid extension products of the target miRNAs, and wherein subsequent to when the denaturation treatment of the polymerase chain reaction is initiated the conditions are adjusted to enable transient hybridization to the 3’ end of the second primary oligonucleotide primers to the 3’ end of the complementary deoxyribonucleic acid extension products, allowing for extension of either one or both the second primary oligonucleotide primers and the complementary deoxyribonucleic acid extension products to form the one or more different primary reverse-transcription/polymerase chain reaction products comprising a 5’ primer-specific portion, a nucleotide sequence portion corresponding to the target miRNA molecule sequence or a complement thereof, a further portion, and a complement of the other 5’ primer-specific portion.

18. The method of claim 1 or claim 2, 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 separated from the quencher molecule;

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, wherein the quencher molecule and the detectable label are cleaved from the one or more unitaq detection probes during the extension treatment and said detecting involves the detection of the cleaved detectable label.

19. The method of claim 3 or claim 4, wherein one primary oligonucleotide primer or one secondary oligonucleotide primer further comprises a unitaq detection portion, thereby forming extension product sequences comprising the 5’ primer-specific portion, the target-specific portions, the unitaq detection portion, and the complement of the other 5’ primer-specific portion, and complements thereof, 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 separated from the quencher molecule;

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

hybridizing the one or more unitaq detection probes to complementary unitaq detection portions on the ligated product sequence or complement thereof during polymerase chain reaction cycles after the first polymerization chain reaction, wherein the quencher molecule and the detectable label are cleaved from the one or more unitaq detection probes during the extension treatment and said detecting involves the detection of the cleaved detectable label.

20. The method of any one of claims 1, 2, or 14, wherein one or both oligonucleotide probes of the oligonucleotide probe set comprises a portion that has no or one nucleotide sequence mismatch when hybridized in a base-specific manner to the target nucleic acid sequence or bisulfite-converted methylated nucleic acid sequence or complement sequence thereof, but have one or more additional nucleotide sequence mismatches that interferes with ligation when said oligonucleotide probe hybridizes in a base-specific manner to a corresponding nucleotide sequence portion in the wildtype nucleic acid sequence or bisulfite-converted unmethylated nucleic acid sequence or complement sequence thereof.

21. The method of claim 1 or claim 2, 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 it complementary target nucleotide sequence of the primary extension product, thereby liberating a 3’OH on the first oligonucleotide probe prior to said ligating.

22. The method of claim 21, wherein the one or more first oligonucleotide probe of the oligonucleotide probe set comprises a sequence that differs from the target nucleic acid sequence or bisulfite-converted methylated nucleic acid sequence or complement sequence thereof, said difference is located two or three nucleotide bases from the liberated free 3’OH end.

23. The method of claims 1 or claim 2, 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 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.

24. The method of claim 1 or claim 2, 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 sequence comprising the first, second, and third oligonucleotide probes of a probe set.