Summary

[0004] The present ~~invention~~ disclosure addresses many of the limitations faced by the conventional multiplexing technologies that exist in the prior art and provides more robust, reliable and efficient methods and systems for analyzing nucleic acids including, but not limited to, multiple microRNAs, mRNAs, long-noncoding RNAs (lncRNAs), genomic DNAs, and synthetic RNAs such as siRNA in a single sample. As described herein, methods and compositions described herein are particularly effective in capturing, analyzing or quantifying low abundance target nucleic acids in a biological sample. Methods and compositions described herein may be used to analyze single target nucleic acid or multiple target nucleic acids simultaneously.

[0005] ~~Thus, in one aspect, t~~The present ~~invention~~ disclosure provides methods of detecting target nucleic acid, comprising steps of: a) contacting a sample with one or more capturing probes, each comprising at least one target capturing sequence, under conditions that permit the one or more capturing probes to capture one or more target nucleic acids in the sample; b) amplifying the captured one or more target nucleic acids in a reaction mixture comprising a detectable entity such that the amplified one or more target nucleic acids are labeled with the detectable entity; c) incubating amplification product with a plurality of re-capturing probes such that the amplified one or more target nucleic acids are re-captured by the plurality of the re-capturing probes; and d) detecting signal generated by a detectable entity associated with the re-captured amplified one or more target nucleic acids, wherein the presence and/or abundance of the detectable signal indicates the presence and/or abundance of the one or more target nucleic acids in the sample.

[0005a] In one aspect of the present disclosure, there is provided a method of detecting target nucleic acid, comprising steps of:

a) Contacting a sample with one or more capturing probes, each comprising at least one target capturing sequence, under conditions that permit the one or more capturing probes to capture one or more target nucleic acids in the sample;

b) Amplifying the captured one or more target nucleic acids in a reaction mixture comprising a detectable entity such that the amplified one or more target nucleic acids are labeled with the detectable entity;

c) Incubating amplification product with a plurality of re-capturing probes such that the amplified one or more target nucleic acids are re-captured by the plurality of the re-capturing probes;

d) Detecting signal generated by detectable entity associated with the re-captured amplified one or more target nucleic acids, wherein the presence and/or abundance of the detectable signal indicates the presence and/or abundance of the one or more target nucleic acids in the sample.

[0006] In some embodiments, each of the capturing probes comprises one target capturing sequence and binds specifically to one distinct target nucleic acid. In some embodiments, each of the capturing probes comprises multiple distinct target capturing sequences and binds to multiple distinct target nucleic acids. In some embodiments, each of the re-capturing probes comprises one target capturing sequence and binds specifically to one distinct target nucleic acid. In some embodiments, each of the re-capturing probes comprises multiple distinct target capturing sequences and binds multiple distinct target nucleic acids. In some embodiments, the capturing probes are and re-capturing probes are identical. In some embodiments, the capturing and/or re-capturing probes are associated with a substrate.

[0028] In some embodiments, the reaction mixture comprises multiple primer sets used to amplify multiple distinct target nucleic acids.

[0029] In some embodiments, each target nucleic acid is present at low abundance in the sample.

[0030] In some embodiments, each target nucleic acid represents less than 1% of total nucleic acids in the biological sample. In some embodiments, each target nucleic acid represents less than 0.1% of total nucleic acids in the biological sample. In some embodiments, each target nucleic acid represents less than 1 out of a million of total nucleic acids in the biological sample. In some embodiments, each target nucleic acid represents less than 1 out of 10 million of total nucleic acids in the biological sample.

[0031] ~~In another aspect, t~~The present ~~invention~~ disclosure also provides methods of detecting target nucleic acid, comprising steps of: a) contacting a sample comprising one or more target nucleic acids with a first set of particles bearing a plurality of capturing probes, each comprising at least one target capturing sequence, under conditions that permit the plurality of capturing probes to capture one or more target nucleic acids in the sample; b) amplifying the captured one or more target nucleic acids in a reaction mixture comprising a detectable entity such that the amplified one or more target nucleic acids are labeled with the detectable entity; and c) incubating amplification product with the original set of particles or a second set of particles bearing a plurality of re-capturing probes such that the amplified one or more target nucleic acids are re-captured by the plurality of the re-capturing probes; wherein each particle has one or more probe regions bearing the plurality of capturing or re-capturing probes and one or more encoding regions bearing detectable moieties that give the identity of the capturing or re-capturing probes thereon; and wherein the presence and/or abundance of the detectable signal generated by detectable entity associated with the re-captured amplified one or more target nucleic acids on the second set of particles indicates the presence and/or abundance of the one or more target nucleic acids in the sample.

[0031a] In another aspect of the present disclosure, there is provided a method of detecting target nucleic acid, comprising steps of:

a) contacting a sample comprising one or more target nucleic acids with a first set of particles bearing a plurality of capturing probes, each comprising at least one target capturing sequence, under conditions that permit the plurality of capturing probes to capture one or more target nucleic acids in the sample;

b) amplifying the captured one or more target nucleic acids in a reaction mixture comprising a detectable entity such that the amplified one or more target nucleic acids are labeled with the detectable entity;

c) incubating amplification product with a second set of particles bearing a plurality of re-capturing probes such that the amplified one or more target nucleic acids are re-captured by the plurality of the re-capturing probes;

wherein each particle has one or more probe regions bearing the plurality of capturing or re-capturing probes and one or more encoding regions bearing detectable moieties that give the identity of the capturing or re-capturing probes thereon; and

wherein the presence and/or abundance of the detectable signal generated by detectable entity associated with the re-captured amplified one or more target nucleic acids on the second set of particles indicates the presence and/or abundance of the one or more target nucleic acids in the sample.

[0032] In some embodiments, the method comprises a step of scanning the second set of particles by a flow-through device to detect the presence and/or abundance of the detectable

biological sample. In some embodiments, each target nucleic acid represents less than 0.1% of total nucleic acids in the biological sample. In some embodiments, each target nucleic acid represents less than 1 out of a million of total nucleic acids in the biological sample. In some embodiments, each target nucleic acid represents less than 1 out of 10 million of total nucleic acids in the biological sample.

[0055] ~~In another aspect, t~~The present ~~invention~~ disclosure also provides diagnostic methods comprising a step of detecting one or more target nucleic acids according to any one of the preceding methods.

[0055a] In a further aspect of the present disclosure, there is provided a diagnostic method comprising a step of detecting one or more target nucleic acids as described herein.

[0056] ~~In another aspect, t~~The present ~~invention~~ disclosure also provides kits for detecting target nucleic acid. In some embodiments, the present ~~invention~~ disclosure provides a kit for detecting target nucleic acid, comprising: particles comprising one or more probe regions bearing probes and one or more coding regions bearing detectable moieties that give the identity of the probes thereon, wherein the probes comprise target capturing sequence; a hyrbridization buffer with pre-determined ionic strength, buffered pH, and denaturing reagent (e.g., formamide and/or 2-pyrrolidone); a labeling buffer comprising one or more oligonucleotide adapters specifically designed to serve as sites for polymerase chain reaction priming and/or reverse transcription; and a PCR buffer containing primers, dNTPs, and reaction reagents for amplification of captured targets. In some embodiments, the kit further comprises a reverse transcriptase and a polymerase. In some embodiments, the kit further comprises a polymerase enzyme that has reverse transcriptase activity. In some embodiments, the kit further comprises a ligase enzyme.

[0056a] In yet another aspect of the present disclosure, there is provided a kit for detecting target nucleic acid, comprising:

particles comprising one or more probe regions bearing probes and one or more encoding regions bearing detectable moieties that give the identity of the probes thereon, wherein the probes comprise target capturing sequence;

a hyrbridization buffer with pre-determined ionic strength, buffered pH, and denaturing reagent;

a labeling buffer comprising adapters designed to serve as sites for polymerase chain reaction priming and/or reverse transcription; and

a PCR buffer containing primers, dNTPs, and reagents for amplification of captured targets.

[0057] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0058] Other features, objects, and advantages of the present ~~invention~~ disclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present ~~invention~~disclosure, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the ~~invention~~ present disclosure will become apparent to those skilled in the art from the detailed description.