Summary

[0004] The present 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] The present 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 a first set of particles bearing 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 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 the first set and second set of particles are identical;

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] The present 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.

[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

Other embodiments and equivalents

[0227] While the present disclosures have been described in conjunction with various embodiments and examples, it is not intended that they be limited to such embodiments or examples. On the contrary, the disclosures encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art. Accordingly, the descriptions, methods and diagrams of should not be read as limited to the described order of elements unless stated to that effect.

[0228] Although this disclosure has described and illustrated certain embodiments, it is to be understood that the disclosure is not restricted to those particular embodiments. Rather, the disclosure includes all embodiments that are functional and/or equivalents of the specific embodiments and features that have been described and illustrated.

CLAIMS

1. A method of detecting target nucleic acid, comprising steps of:

a) Contacting a sample with a first set of particles bearing 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 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 recapturing probes, wherein the first set and second set of particles are identical;

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.

2. The method of claim 1, wherein the particles are made of a material selected from the group consisting of hydrogel, glass, photoresists, silica, polystyrene, polyethylene glycol, agarose, chitosan, alginate, PLGA, optical fiber, cellulose, and combination thereof.

3. The method of claim 1 or claim 2, wherein the capturing or re-capturing probes are embedded throughout one or more probe regions of the particle, optionally wherein the particle further comprises one or more encoding regions and wherein the one or more encoding regions bear detectable moieties that give the identity of the capturing or re-capturing probes.

4. The method of any one of the preceding claims, wherein the one or more target nucleic acids are microRNAs, mRNAs, non-coding transcripts, genomic DNA, cDNAs, siRNAs, DNA/RNA chimera, or combination thereof.

5. The method of any one of the preceding claims, wherein the probe is DNA, RNA, DNA/RNA chimera, or combination thereof, optionally wherein the probe is specific to the target nucleic acid comprises a target capture sequence that is substantially complementary to the target nucleic acid.

6. The method of any one of the preceding claims, wherein the method further comprises a step of coupling one or more adapters to the captured target nucleic acid,

optionally wherein the one or more adapters are universal adapters, optionally wherein the one or more adapters are coupled to the target nucleic acid at the 3'-terminus, the 5'terminus, or both the 3'-terminus and 5'-terminus, optionally wherein the one or more adapters are DNA, RNA, DNA/RNA chimera, or combination thereof, optionally wherein the captured target nucleic acid is first digested by a nuclease or restriction enzyme to remove single-stranded 5' and or 3' regions prior to the coupling of the one or more adapters, optionally wherein each of the capturing probes further comprises sequences complementary to the one or more adapters, optionally wherein the sequences complementary to the one or more adapters are adjacent to the target capture sequence, optionally wherein the one or more adapters are coupled to the target nucleic acid via ligation, optionally wherein the ligation is performed by a DNA or RNA ligase enzyme, optionally wherein the one or more adapters comprise sequences specifically designed to serve as sites for polymerase chain reaction priming, reverse transcription, or modification by other DNA-modifying or RNA-modifying enzymes.

7. The method of any one of the preceding claims, wherein
(i) the step of amplifying the captured target nucleic acid comprises performing a polymerase chain reaction (PCR), optionally wherein the PCR reaction uses polymerase enzyme selected from Taq, Bst, and/or Phi29,

optionally wherein the PCR is performed

(a) with a single primer set, optionally wherein the PCR is performed with one primer; and/or

(b) with primers attached to the substrate; and/or

(c) using a combination of universal, specific, or poly(A) primers; and/or

(ii) the captured target is reverse transcribed prior to amplification, optionally wherein reverse transcription is catalyzed by a polymerase enzyme with reverse transcriptase activity, optionally wherein the polymerase enzyme is Pyrophage or TtH, optionally wherein reverse transcription is catalyzed by one enzyme and PCR amplification is carried out by a second enzyme; and/or

(iii) the step of amplifying the captured target nucleic acid is performed isothermally;

and/or

(vi) the target nucleic acid and/or the one or more adapters are circularized via ligation or enzymatic polymerization.

8. The method of any one the preceding claims, wherein the detectable entity is (i) selected from the group consisting of fluorophores, dye, biotin, radioisotopes, antibodies, aptamers, polypeptides, quantum dots, chromophores; and/ or (ii) provided in the reaction mixture as labeled primer, labeled dNTPs and/or intercalating dye.

9. The method of any one of the preceding claims, wherein
(i) the captured one or more target nucleic acids are separated from the capturing probes prior to amplification, optionally wherein the captured one or more target nucleic acids are separated from the capturing probes by denaturation using heat, chemical denaturants, or a helicase enzyme; and/or
(ii) the substrate is present during the time of amplification; and/or
(iii) the step of amplifying the captured one or more target nucleic acids is performed using a single primer or less than 5 primer pairs.

10. The method of any one of claims 7-9, wherein the PCR is biased such that a substantial fraction of the amplified one or more target nucleic acids is single-stranded, optionally wherein the PCR is biased towards single-stranded amplified target nucleic acid through (i) designing a forward primer with a significantly lower annealing temperature than a reverse primer, or (ii) adding the forward primer at a concentration such that it is exhausted during the PCR reaction, optionally wherein the ratio between the forward primer and the reverse primer is less than 1:2.

11. The method of any one of the preceding claims, wherein
(i) the amplification product and the plurality of re-capturing probes are incubated under stringent hybridization condition; and/or

(ii) the re-capturing of amplified one or more target nucleic acids are performed under substantially more stringent conditions than the capturing step; and/or
(iii) the substrate is rinsed between steps to remove unbound probes, target nucleic acids and/or adapters; and/or
(iv) the reaction mixture comprises (a) a single primer set used to amplify multiple distinct target nucleic acids or (b) multiple primer sets used to amplify multiple distinct target nucleic acids; and/or
(v) the conditions are tuned in order to give stringent capture by controlling:
temperature, time, monovalent salt concentration, divalent salt concentration, dNTP concentration, or the addition ofDMSO, formamide, polyethylene glycol, 2-pyrrolidone, or other agents that alter the kinetics of DNA duplex formation.

12. The method of any one of the preceding claims, wherein the sample is a biological sample, optionally wherein the biological sample is a preparation of isolated DNA or RNA, protease tissue digest, cell lysate, serum, plasma, whole blood, urine, stool, saliva, cord blood, chorionic villus sample, chorionic villus sample culture, amniotic fluid, amniotic fluid culture, transcervical lavage fluid, and combination thereof.

13. The method of any one of the preceding claims, wherein the signal generated by detectable entity is detected by a flow cytometer, or array scanner, optionally wherein the signal is quantified.

14. The method of any one of the preceding claims, wherein the one or more capturing probes comprises multiple capturing probes specific to multiple target nucleic acids, optionally wherein the multiple probes are associated with multiple particles, with each particle comprising probes specific to same target nucleic acid, optionally wherein
(i) the each particle is encoded to provide identity of the specific probes thereon,

wherein the each particle is encoded through incorporation of one or more fluorophores with known spectral characteristics; or (ii) the multiple capturing probes are located on multiple distinct regions of a planar substrate.

15. The method of any one of the preceding claims, wherein each target nucleic acid represents less than 1 %, or less than 0.1 %, less than 1 out of a million, or less than 1 out of 10 million of total nucleic acids in the biological sample.

16. A diagnostic method comprising a step of detecting one or more target nucleic acids according to any one of the preceding claims.

17. 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.

18. The kit of claim 17, wherein the kit comprises a ligase, optionally wherein the kit further comprises (i) a reverse transcriptase and a polymerase

or (ii) a polymerase with reverse transcriptase activity; and/or the denaturing reagent is formamide and/or 2-pyrrolidone.