CLAIMS

1. A method of amplification of a target nucleic acid, the method comprising annealing the target nucleic acid and a control nucleic acid in the presence of a reverse primer of the target nucleic acid and the control nucleic acid,

subjecting the target nucleic acid and the control nucleic acid to reverse transcription,

subjecting the annealed reverse transcribed target nucleic acid and the control nucleic acid to two or more amplification steps,

wherein the first amplification step is an emulsion-based polymerase chain reaction (PCR) in the presence of a surfactant and an amplification mixture and

the second or more amplification step is a CoT PCR in the presence of an amplification mixture,

wherein the amplification mixture for the first and second or more amplification steps comprises an RNase and an oligonucleotide primer and/or probe capable of hybridizing with the reverse transcribed target nucleic acid and the control nucleic acid, wherein the oligonucleotide primer and/or probe comprises an RNase-dependent PCR primer (rhPCR primer), and

wherein the second or more amplification steps comprises interposing a hybridization step between denaturation and annealing, and the control nucleic acid is included in greater abundance than the target nucleic acid.

2. The method of claim 1, wherein the first amplification step of the nucleic acid is performed in the presence of three parts surfactant to one part amplification mixture.

3. The method of any one of claims 1 to 2, wherein the method further comprises a step of freezing and thawing the amplified mixture between the first amplification step and the second or more amplification steps

4. The method of any one of claims 1 to 3, wherein the oligonucleotide primer and/or probe capable of hybridizing with the target nucleic acid comprises a 5’ end of a functional primer, a cleavage site, one or more matching DNA bases, and one or more mismatch DNA base with one or more blocking group at the 3’ end.

5. The method of any one of claims 1 to 4, wherein the cleavage site is one or more RNA residues.

6. The method of any one of claims 1 to 5, wherein the oligonucleotide primer and/or probe capable of hybridizing with the target nucleic acid comprises a 5' end of a functional primer, a cleavage site consisting of one or more RNA residue, one or more matching DNA bases, and one or more mismatch DNA base with one or more blocking group at the 3' end.

7. The method of any one of claims 1 to 6, wherein the control nucleic acid is added to a sample at a constant amount to thereby normalize amplification efficiency across a plurality of samples.

8. The method of any one of claims 1 to 7, wherein the control nucleic acid is added to the sample at 10^2 to 10^{10} copies.

9. The method of any one of claims 1 to 8, wherein the target nucleic acid is a cell free nucleic acid.

10. The method of claim 9, wherein the cell free nucleic acid is a circulating cell free nucleic acid.

11. The method of any one of claims 1 to 10, wherein the target nucleic acid is a cell free RNA.

12. The method of claim 11, wherein the cell free RNA is a circulating cell free RNA.

13. The method of any one of claims 1 to 12, wherein the target nucleic acid is obtained from a biological sample.

14. The method of any one of claims 1 to 13, wherein the method is a real time amplification method.

15. A method of detecting and/or determining the presence and/or the amount of a target nucleic acid in a sample comprising

annealing the target nucleic acid and a control nucleic acid in the presence of a reverse primer of the target nucleic acid and the control nucleic acid,

subjecting the target nucleic acid and the control nucleic acid to reverse transcription,

subjecting the reverse transcribed target nucleic acid and the control nucleic acid to two or more amplification steps,

wherein the first amplification step is an emulsion-based polymerase chain reaction (PCR) in the presence of a surfactant and an amplification mixture and

the second or more amplification step is a CoT PCR in the presence of an amplification mixture,

wherein the amplification mixture for the first and second or more amplification steps comprises an RNase, an oligonucleotide capable of hybridizing with the target nucleic acid and the control nucleic acid, wherein the oligonucleotide primer and/or probe comprises an RNase-dependent PCR primer (rhPCR primer) , and

wherein the second or more amplification steps comprises interposing a hybridization step between denaturation and annealing,

and the control nucleic acid is included in greater abundance than the target nucleic acid,

wherein the method further comprises quantifying the amount of target nucleic acid present in the sample and/or sequencing the target nucleic acid in the sample.