CLAIMS

1. A method of detecting and/or determining the presence of one or more thyroid-specific circulating cell-free RNA (cfRNA), the method comprising

annealing the one or more thyroid-specific cfRNA and a control cfRNA in the presence of a reverse primer of the one or more thyroid-specific cfRNA and the control cfRNA,

subjecting the one or more thyroid-specific cfRNA and the control cfRNA to reverse transcription,

subjecting each of the annealed one or more thyroid-specific cfRNA and the control cfRNA to two or more amplification steps,

wherein the first amplification step is in the presence of a surfactant and an amplification mixture,

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

wherein the amplification mixture for the first and second or more amplification steps comprises an oligonucleotide primer and/or probe capable of hybridizing with the one or more thyroid-specific cfRNA and the control cfRNA, wherein the oligonucleotide primer and/or probe comprises a cleavage site, and a cleavable 3' end, and an RNase, and

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

2. The method of claim 1, wherein the amplification step of the cfRNA 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 freeze and thawing the amplified mixture.

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

5. The method of any one of claims 1 to 4, wherein the control cfRNA is added to the sample at a constant amount to thereby normalizes of the amplification efficiency across a plurality of samples.

6. The method of any one of claims 1 to 5, wherein the control cfRNA is added to the sample at about 10^2 to 10^{10} copies.

7. The method of any one of claims 1 to 5, wherein the method further comprises a step of quantifying the amount of one or more thyroid-specific cfRNA present in the sample and/or sequencing the target cfRNA in the sample.

8. The method of any one of claims 1 to 6, wherein the one or more thyroid-specific cfRNA is obtained from a biological sample.

9. The method of any one of claims 1 to 8, wherein the thyroid-specific nucleic acid are genes that are highly expressed and/or have four or more folds-change expression in the thyroid as compared to in other tissues.

10. The method of any one of claims 1 to 9, wherein the one or more thyroid-specific cfRNA comprise thyroid peroxidase (TPO), thyroglobulin (TG), GFRA2 (glial cell line-derived neurotrophic factor family receptor alpha-2), IYD (iodotyrosine deiodinase), PDE8B (phosphodiesterase-8B), WDR86 (WD repeat domain 86), C16orf89 (Chromosome 16 Open Reading Frame 89), DGKI (Diacylglycerol kinase), DIO2 (Iodothyronine Deiodinase 2), TSHR (Thyroid Stimulating Hormone Receptor), and PAX8 (Paired box gene 8).

11. The method of any one of claims 1 to 10, wherein the one or more thyroid-specific cfRNA comprises thyroid peroxidase (TPO), GFRA2 (glial cell line-derived neurotrophic factor family receptor alpha-2), IYD (iodotyrosine deiodinase), and thyroglobulin (TG).

12. The method of any one of claims 1 to 11, wherein the sample is obtained from a subject prior to and/or subsequent to a treatment.

13. The method of any one of claims 1 to 11, wherein the treatment is a surgery to remove thyroid and/or a radiation therapy.

14. The method of any one of claims 12 and 13,

wherein the method comprises detecting and/or determining and/or quantifying the presence of the one or more thyroid-specific gene in a first sample and a second sample,

wherein the first sample is taken at an earlier time point than the second sample, and

wherein an increase in the presence of the one or more thyroid-specific gene in the second sample compared to the first sample indicates the subject to have residual thyroid tissue and/or tumour burden,

wherein an increase in the presence of the one or more thyroid-specific gene in the second sample compared to the first sample indicates the subject to have recurrence and/or metastasis of thyroid conditions, and

wherein the sample is a plasma sample.

15. A method of detecting and/or determining thyroid cancer recurrence and/or metastasis in one or more samples obtained from a subject in need thereof, the method comprising

annealing the one or more thyroid-specific cfRNA and a control cfRNA in the presence of a reverse primer of the one or more thyroid-specific cfRNA and the control cfRNA, and

subjecting the one or more thyroid-specific cfRNA and the control nucleic acid to reverse transcription,

subjecting each of the annealed one or more thyroid-specific cfRNA and the control cfRNA to two or more amplification steps,

wherein the first amplification step is in the presence of a surfactant and an amplification mixture comprising and

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

wherein the amplification mixture for the first and second or more amplification steps comprises an oligonucleotide primer and/or probe capable of hybridizing with the one or more thyroid-specific cfRNA and the control nucleic acid, wherein the oligonucleotide primer and/or probe comprises a cleavage site, and a cleavable 3’ end, and RNase, and

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

wherein the method comprises detecting and/or determining and/or quantifying the presence of the one or more thyroid-specific gene in a first sample and a second sample,

wherein the first sample is taken at an earlier time point than the second sample,

wherein an increase in the presence of the one or more thyroid-specific gene in the second sample compared to the first sample indicates the subject to have thyroid cancer recurrence and/or metastasis, and

wherein the sample is a plasma sample.

16. The method of claim 15, wherein the one or more thyroid-specific gene comprises thyroid peroxidase (TPO), sodium-iodide symporter (NIS), thyroglobulin (TG), and thyroid stimulating hormone receptor (TSHR).