

CLAIMS

1. A method of determining the expression level of a specific nucleic acid molecule in a biological sample, the method comprising the steps of:

5 (i) providing a batch A comprising DNA or cDNA isolated/obtained from the biological sample;

(ii) providing three or more aliquots of batch A provided in step (i) and performing an independent polymerase chain reaction (PCR) with each of the three or more aliquots in order to amplify the specific nucleic acid molecule, thereby providing three or more batches B comprising the amplified specific nucleic acid molecule; and

10 (iii) mixing equal amounts of the three or more batches B, thereby providing a batch C, and determining the level of the specific nucleic acid molecule in batch C by a PCR-based approach,

in which the level determined in step (iii) corresponds to the expression level of the specific nucleic acid molecule in the biological sample.

15 2. A method of determining the expression level of a specific nucleic acid molecule in a biological sample, the method comprising the steps of:

(i) providing a batch A comprising DNA or cDNA isolated/obtained from the biological sample;

20 (ii) providing three or more aliquots of batch A provided in step (i) and performing an independent polymerase chain reaction (PCR) with each of the three or more aliquots in order to amplify the specific nucleic acid molecule, thereby providing three or more batches B comprising the amplified specific nucleic acid molecule; and

(iii) determining the level of the specific nucleic acid molecule in each of the three or more batches B by a PCR-based approach,

25 in which the mean value of the levels determined in step (iii) corresponds to the expression level of the specific nucleic acid molecule in the biological sample.

3. A method according to Claim 1 or 2, in which the concentration of the specific nucleic acid molecule in the biological sample is $\leq 1 \times 10^{-11}$ M, or $\leq 1 \times 10^{-12}$ M, or $\leq 1 \times 10^{-13}$ M, or $\leq 1 \times 10^{-14}$ M, or $\leq 1 \times 10^{-15}$ M, or $\leq 1 \times 10^{-16}$ M.
4. A method according to Claim 3, in which the concentration of the specific nucleic acid molecule in the biological sample is between 1×10^{-11} M and 1×10^{-17} M, or 1×10^{-12} M and 1×10^{-17} M, or 1×10^{-13} M and 1×10^{-17} M, or 1×10^{-14} M and 1×10^{-17} M, or 1×10^{-15} M and 1×10^{-17} M, or 1×10^{-16} M and 1×10^{-17} M.
5. A method according to any preceding claim, in which the specific nucleic acid molecule is selected from the group consisting of a specific miRNA, a specific cell-free circulating DNA (e.g., a specific cell-free circulating tumor DNA), a specific mRNA, a specific siRNA and a specific snRNA.
6. A method according to Claim 5, in which the specific nucleic acid molecule is a specific miRNA.
7. A method according to Claim 6, in which the specific miRNA molecule is selected from the group consisting of miR-371a-3p, miR-93-5p, miR-372, miR-373, miR-367 and miR-20a-5p.
8. A method according to any preceding claim, in which the biological sample is selected from the group consisting of body fluid, tissue, cells, cell lysate and cell culture supernatant.
9. A method according to Claim 8, in which the body fluid is selected from the group consisting of blood serum, blood plasma, seminal plasma, hydrocele fluid, spermatocele fluid, whole blood, urine, amniotic fluid, exudate, sputum, saliva and cerebrospinal fluid.
10. A method according to Claim 8, in which the tissue is selected from the group consisting of native tissue, snap-frozen tissue and formalin-fixed and paraffin-embedded (FFPE) tissue.
11. A method according to Claim 8 or 10, in which the tissue is tumor tissue.
12. A method according to any preceding claim, in which batch A comprises cDNA isolated/obtained from the biological sample.
13. A method according to Claim 12, in which step (i) comprises the steps of:
 - (ia) isolating RNA from the biological sample; and

(ib) converting the RNA isolated in step (ia) into cDNA, thereby providing batch A comprising the cDNA.

14. A method according to any preceding claim, in which, in step (ii), three aliquots of batch A are provided.

5 15. A method according to any preceding claim, in which the PCR-based approach is quantitative real-time PCR (qRT-PCR) or digital PCR (dPCR).

16. A method of detecting a disease or disorder in a subject or of determining the extent/progression of a disease or disorder in a subject, the method comprising determining the expression level of a specific nucleic acid molecule in a biological sample obtained from
10 the subject with a method according to any preceding claim, in which the expression level of the specific nucleic acid molecule in the biological sample is indicative of the presence, absence and/or extent/progression of the disease or disorder in the subject.