

SUMMARY

[0004] In one aspect, a computer-implemented method for quantitation of biological material in a biological sample is provided. The method includes receiving amplification data from amplification of a first and a second reference sample using PCR (Polymerase Chain Reaction) and receiving amplification data from amplification of a biological sample using PCR. The method further includes determining a specific PCR efficiency from the received amplification data from amplification of the first and second reference sample. The method includes determining a relative PCR efficiency for the biological sample. Next, the method includes determining a quantity of biological material in the biological sample using the relative PCR efficiency, wherein the relative PCR efficiency is determined with the following equation:

$$\epsilon_{S2A2} = \epsilon_{S1A1}^{\frac{CT_{100\%S1A1}}{CT_{100\%S2A2}}},$$

wherein S1A1 represents a first assay for a reference gene, S2A2 represents a second assay for a target gene, $CT_{100\%S1A1}$ represents a cycle threshold of the first assay, $CT_{100\%S2A2}$ represents a cycle threshold in the second assay, ϵ_{S1A1} is the specific PCR efficiency of the first assay, and ϵ_{S2A2} is the specific PCR efficiency of the second assay.

[0005] In one aspect, there is provided a computer-readable storage medium encoded with processor-executable instructions for quantitation of biological material in a biological sample, the instructions comprising instructions for: receiving amplification data from amplification of a first and a second reference sample using PCR (Polymerase Chain Reaction); receiving amplification data from amplification of a biological sample; determining an efficiency from the received amplification data from amplification of the first and second reference sample; determining a relative PCR efficiency for the biological sample; and determining a quantity of biological material in the biological sample using the relative PCR efficiency, wherein the relative PCR efficiency is determined with the following equation:

$$\epsilon_{S2A2} = \epsilon_{S1A1}^{\frac{CT_{100\%S1A1}}{CT_{100\%S2A2}}},$$

wherein S1A1 represents a first assay for a reference gene, S2A2 represents a second assay for a target gene, CT100%S1A1 represents a cycle threshold of the first assay, CT100%S2A2 represents a cycle threshold in the second assay, ϵ S1A1 is the specific PCR efficiency of the first assay, and ϵ S2A2 is the specific PCR efficiency of the second assay. In another aspect, there is provided a system for quantitation of biological material in a biological sample, the system comprising: a processor; and a memory configured to store processor-executable instructions for: receiving amplification data from amplification of a first and a second reference sample using PCR (Polymerase Chain Reaction); receiving amplification data from amplification of a first and a second reference sample; receiving amplification data from amplification of a biological sample; determining an efficiency from the received amplification data from amplification of the first and second reference sample; determining a relative PCR efficiency for the biological sample; and determining a quantity of biological material in the biological sample using the relative PCR efficiency, wherein the relative PCR efficiency is determined with the following equation:

$$\epsilon_{S2A2} = \epsilon_{S1A1}^{\frac{CT_{100\%S1A1}}{CT_{100\%S2A2}}},$$

wherein S1A1 represents a first assay for a reference gene, S2A2 represents a second assay for a target gene, CT100%S1A1 represents a cycle threshold of the first assay, CT100%S2A2 represents a cycle threshold in the second assay, ϵ S1A1 is the specific PCR efficiency of the first assay, and ϵ S2A2 is the specific PCR efficiency of the second assay.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] FIG. 1 illustrates a workflow comparing current quantitation methods and quantitation methods according to various embodiments described herein;

[0007] FIG. 2 illustrates a method of quantitation according to various embodiments described herein;

[0008] FIG. 3 illustrates a method of quantitation according to various embodiments described herein;

[0009] FIG. 4 illustrates an exemplary plate layout method of quantitation according to various embodiments described herein;

[0010] FIG. 5 illustrates an exemplary scattered plot of calculated %C using a traditional standard curve Vs. Input %C;

[0011] FIG. 6 illustrates an exemplary scattered plot of calculated %C not using standard curve Vs. Input %C according to various embodiments described herein;

CLAIMS

1. A computer-implemented method for quantitation of biological material in a biological sample, the method comprising:

receiving amplification data from amplification of a first and a second reference sample using PCR (Polymerase Chain Reaction);

receiving amplification data from amplification of a biological sample using PCR;

determining a specific PCR efficiency from the received amplification data from amplification of the first and second reference sample;

determining a relative PCR efficiency for the biological sample; and

determining a quantity of biological material in the biological sample using the relative PCR efficiency, wherein the relative PCR efficiency is determined with the following equation:

$$\varepsilon_{S2A2} = \varepsilon_{S1A1}^{\frac{CT_{100\%S1A1}}{CT_{100\%S2A2}}},$$

wherein S1A1 represents a first assay for a reference gene, S2A2 represents a second assay for a target gene, $CT_{100\%S1A1}$ represents a cycle threshold of the first assay, $CT_{100\%S2A2}$ represents a cycle threshold in the second assay, ε_{S1A1} is the specific PCR efficiency of the first assay, and ε_{S2A2} is the specific PCR efficiency of the second assay.

2. The method of claim 1, comprising the step of amplification of a first and a second reference sample, wherein a first and a second state are defined for the respective first and second reference sample, and wherein the amplification of the first and second reference sample is performed with a single primer assay specific for either of the two states.

3. The method of claim 1, wherein the quantity of biological material relates to a methylation level of the biological sample.

4. The method of claim 3, wherein the methylation level of the biological sample is determined by the following equation:

$$x\% \approx E_{S1A1}^{(CT_{R1}-CT_1)} \times 100\%,$$

wherein $x\%$ is the methylation level of the biological sample, CT_{R1} is a reference cycle threshold of a reference sample having a methylation level of 100%, and CT_1 is a cycle threshold of the biological sample.

5. The method of claim 1, wherein the quantity of biological material relates to determining a genotyping result.

6. The method of claim 1, wherein the quantity of biological material relates to determining a copy number variation result.

7. The method of claim 1, further comprising:
receiving amplification data from amplification of a third reference sample; and
determining the efficiency from amplification of the first, second, and third reference sample.

8. The method of claim 7, wherein a first and a second state are defined for the respective first and second reference sample, and wherein the amplification of the first, second, and third reference sample is performed with a dual primer assay specific for both states.

9. A computer-readable storage medium encoded with processor-executable instructions for quantitation of biological material in a biological sample, the instructions comprising instructions for:

receiving amplification data from amplification of a first and a second reference sample using PCR (Polymerase Chain Reaction);
receiving amplification data from amplification of a biological sample;
determining an efficiency from the received amplification data from amplification of the first and second reference sample;
determining a relative PCR efficiency for the biological sample; and
determining a quantity of biological material in the biological sample using the relative PCR efficiency, wherein the relative PCR efficiency is determined with the following equation:

$$\mathcal{E}_{S2A2} = \mathcal{E}_{S1A1}^{\frac{CT_{100\%S1A1}}{CT_{100\%S2A2}}}$$

wherein S1A1 represents a first assay for a reference gene, S2A2 represents a second assay for a target gene, $CT_{100\%S1A1}$ represents a cycle threshold of the first assay, $CT_{100\%S2A2}$ represents a cycle threshold in the second assay, $\mathcal{E}_{S1A1}$ is the specific PCR efficiency of the first assay, and $\mathcal{E}_{S2A2}$ is the specific PCR efficiency of the second assay.

10. The computer-readable storage medium of claim 9, wherein a first and a second state are defined for the respective first and second reference sample, and wherein the amplification of the first and second reference sample is performed with a single primer assay specific for either of the two states.

11. The computer-readable storage medium of claim 9, wherein the quantity of biological material relates to a methylation level of the biological sample.

12. The computer-readable storage medium of claim 11, wherein the methylation level of the biological sample is determined by the following equation:

$$x\% \approx \mathcal{E}_{S1A1}^{(CT_{R1}-CT_1)} \times 100\%,$$

wherein x% is the methylation level of the biological sample, CT_{R1} is a reference cycle threshold of a reference sample having a methylation level of 100%, and CT_1 is a cycle threshold of the biological sample.

13. The computer-readable storage medium of claim 9, wherein the quantity of biological material relates to determining a genotyping result.

14. The computer-readable storage medium of claim 9, wherein the quantity of biological material relates to determining a copy number variation result.

15. The computer-readable storage medium of claim 9, wherein the instructions further comprise instructions for:

receiving amplification data from amplification of a third reference sample; and

determining the efficiency from amplification of the first, second, and third reference sample.

16. The computer-readable storage medium of claim 15, wherein a first and a second state are defined for the respective first and second reference sample, and wherein the amplification of the first, second, and third reference sample is performed with a dual primer assay specific for both states.

17. A system for quantitation of biological material in a biological sample, the system comprising:

a processor; and

a memory configured to store processor-executable instructions for:

receiving amplification data from amplification of a first and a second reference sample using PCR (Polymerase Chain Reaction);

receiving amplification data from amplification of a biological sample;

determining an efficiency from the received amplification data from amplification of the first and second reference sample;

determining a relative PCR efficiency for the biological sample; and

determining a quantity of biological material in the biological sample using the relative PCR efficiency, wherein the relative PCR efficiency is determined with the following equation:

$$\mathcal{E}_{S2A2} = \mathcal{E}_{S1A1}^{\frac{CT_{100\%S1A1}}{CT_{100\%S2A2}}},$$

wherein S1A1 represents a first assay for a reference gene, S2A2 represents a second assay for a target gene, $CT_{100\%S1A1}$ represents a cycle threshold of the first assay, $CT_{100\%S2A2}$ represents a cycle threshold in the second assay, $\mathcal{E}_{S1A1}$ is the specific PCR efficiency of the first assay, and $\mathcal{E}_{S2A2}$ is the specific PCR efficiency of the second assay.

18. The system of claim 17, wherein a first and a second state are defined for the respective first and second reference sample, and wherein the amplification of the first and

second reference sample is performed with a single primer assay specific for either of the two states.

19. The system of claim 17, wherein the quantity of biological material relates to a methylation level of the biological sample.

20. The system of claim 19, wherein the methylation level of the biological sample is determined by the following equation:

$$x\% \approx \varepsilon_{S1A1}^{(CT_{R1}-CT_1)} \times 100\%,$$

wherein x% is the methylation level of the biological sample, CT_{R1} is a reference cycle threshold of a reference sample having a methylation level of 100%, and CT₁ is a cycle threshold of the biological sample.

21. The system of claim 17, wherein the quantity of biological material relates to determining a genotyping result.

22. The system of claim 17, wherein the quantity of biological material relates to determining a copy number variation result.

23. The system of claim 17, wherein the instructions further comprise instructions for:
receiving amplification data from amplification of a third reference sample; and
determining the efficiency from amplification of the first, second, and third reference sample.

24. The system of claim 23, wherein a first and a second state are defined for the respective first and second reference sample, and wherein the amplification of the first, second, and third reference sample is performed with a dual primer assay specific for both states.