SYSTEMS AND METHODS FOR MODEL-BASED qPCR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 61/322,895, filed April 11, 2010, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Polymerase Chain Reaction (PCR) instrumentation has made it possible to perform reliable quantification of DNA or RNA levels in biological samples. Commercially available PCR instruments, and related data acquisition and analysis software, process qPCR assay data generated from biological samples. These systems report quantitative results by calculating a threshold cycle (C_T or C_{RT}) value as the fractional PCR cycle number where the reporter signal rises above a threshold set manually by a human or automatically by software. The determined C_T value can be used to estimate the initial quantity of DNA material.

[0003] Various approaches for the analysis of PCR amplification curves known in the art may rely, at least in part, on defining a linear exponential range for a portion of a PCR amplification curve in order to determine a cycle threshold for a sample. Approaches using modeling of a PCR amplification curve to determine a cycle threshold value for a sample may be difficult to use successfully, due to the complexity of modeling such curves. Various models for a PCR amplification curve require at least about 15 parameters or more in order to approximately model a PCR amplification curve. Furthermore, the beginning and later points in an amplification curve may be noisy and insensitive to parameter change.

SUMMARY

[0004] According to an aspect of the present invention~~In one exemplary embodiment~~, a method for determining a cycle threshold for a PCR amplification curve is provided. The method includes receiving a data set for a plurality of biological samples for a PCR amplification reaction. The data set includes a plurality of amplification curves, each amplification curve associated with a biological sample of the plurality of biological samples. The method further includes performing a nonlinear optimization comprising a fit of each amplification curve to a ~~complementary~~ modeled amplification curve to determine a best-fit set of parameters for a modeled efficiency curve and ~~associated~~the amplification curve. The

modeled amplification curve is based on a modeled efficiency curve. The method includes determining a cycle threshold value for each biological sample based on a complementary relationship of the modeled efficiency curve to the modeled amplification curve. In various embodiments, the nonlinear optimization is a constrained nonlinear optimization.

[0004a] According to another aspect of the present invention, there is provided a computer-readable medium encoded with instructions, executable by a processor, for determining a cycle threshold for a PCR amplification curve, the instructions comprising instructions for: receiving a data set for a plurality of biological samples for a PCR amplification reaction, wherein the data set includes a plurality of amplification curves, each amplification curve associated with a biological sample of the plurality of biological samples; performing a nonlinear optimization comprising a fit of each amplification curve to a modeled amplification curve to determine a best-fit set of parameters for a modeled efficiency curve and the amplification curve, wherein the modeled amplification curve is based on a modeled efficiency curve; and determining a cycle threshold value for each biological sample based on a complementary relationship of the modeled efficiency curve to the modeled amplification curve.

[0004b] According to another aspect of the present invention, there is provided a system for determining a cycle threshold for a PCR amplification curve, the system comprising: a processor; and a memory storing instructions executable by the processor, the instructions comprising instructions for: receiving a data set for a plurality of biological samples for a PCR amplification reaction, wherein the data set includes a plurality of amplification curves, each amplification curve associated with a biological sample of the plurality of biological samples; performing a nonlinear optimization comprising a fit of each amplification curve to a modeled amplification curve to determine a best-fit set of parameters for a modeled efficiency curve and the amplification curve, wherein the modeled amplification curve is based on a modeled efficiency curve; and determining a cycle threshold value for each biological sample based on a complementary relationship of the modeled efficiency curve to the modeled amplification curve.