

WHAT IS CLAIMED IS:

1 1. A method of determining one or more properties of a biological and/or
 2 chemical reaction from a data set representing an amplification process of the reaction, the
 3 method comprising:
 4 receiving a set of data points representing a curve having a baseline portion and a
 5 growth portion, each data point representing a physical quantity of a substance during an
 6 amplification process;
 7 calculating, with a processor, a first function that approximates the set of data
 8 points;
 9 extracting one or more parameters from the first function;
 10 using the one or more parameters to calculate, with the processor, a second
 11 function that approximates the set of data points; and
 12 determining one or more properties of the biological and/or chemical reaction
 13 using the second function.

1 2. The method of claim 1, wherein the one or more properties include a
 2 quantitation value associated with the amplification process.

1 3. The method of claim 1, wherein the one or more parameters are used as
 2 initial values for the second function, and wherein calculating the second function includes
 3 changing one or more the initial values to reduce an error between the second function and the
 4 set of data points.

1 4. The method of claim 3, wherein the second function is a modified sigmoid
 2 function that contains a drift term.

1 5. The method of claim 4, wherein the modified sigmoid function is
 2 represented by: $f = a_0 + \frac{1}{D} \left[a_1 + \left(\frac{a_4}{D} \right) \ln \left(\frac{D}{h} \right) \right]$, where $D = 1 + h$, $h = \exp(E)$, $E = \frac{(a_2 - x)}{a_3}$,
 3 and $\alpha = \frac{2a_4}{a_1}$, where a_0 , a_1 , a_2 , a_3 , and a_4 are numbers determined during a fitting procedure of

the modified sigmoid function to the set of data points, and where x is a cycle number of the amplification process.

6. The method of claim 5, wherein the one or more parameters include a_0 , a_1 , a_2 , and a_3 .

7. The method of claim 1, wherein the one or parameters includes a time C_t in the amplification process at which the first function has a specified value.

8. The method of claim 7, wherein using the time C_t to calculate a second function that approximates the set of data points includes:
defining a window of time centered at C_t ;
using the data points within the time window to determine the second function such that the second function approximates the data points within the time window.

9. The method of claim 8, wherein the second function is a polynomial of sixth order or higher.

10. The method of claim 7, wherein the time C_t is determined as a weighted average of a first time where a second derivative of the first function has a maximum value and a second time where a third derivative of the first function has a maximum value.

11. The method of claim 1 further comprising:
selecting an end of the baseline portion as a first time of the amplification process, wherein an integrated area of a first derivative function of the first function from the first time to a peak of the first derivative function is within a range of specified values.

12. The method of claim 1 further comprising:
analyzing the first function to determine whether a slope of the first function exceeds a maximum amplification slope; and
if the first function does have locations where the maximum amplification slope is exceeded, then treating segments containing the locations as a non-amplifying segment of the amplification curve.

1 13. The method of claim 12 wherein the maximum amplification slope is
2 $\ln(2.0)$ multiplied by a value of a data point at a location where the slope is being calculated.

1 14. A method of determining a baseline region for an amplification curve
2 resulting from an amplification process of a biological and/or chemical reaction, the method
3 comprising:

4 receiving a set of data points representing a curve having a baseline portion and a
5 growth portion, each data point representing a physical quantity of a substance during an
6 amplification process;

7 determining, with a processor, a function that approximates the set of data points;

8 calculating a first derivative of the function to obtain a first derivative function;

9 determining an end of a baseline region by:

10 for a plurality of points:

11 integrating, with the processor, the first derivative function from the
12 respective point to a fixed location of the first derivative function to obtain a respective
13 integrated area; and

14 selecting a point whose integrated area is within a specified range as the end
15 of the baseline region; and

16 determining a beginning of the baseline region.

1 15. The method of claim 14, wherein the first derivative function includes the
2 first derivative of the function subtracted by the first derivative at an initial data point.

1 16. The method of claim 15, wherein the integrated area of the selected point
2 is a predetermined percentage of the area the first derivative function from the initial point to the
3 fixed location.

1 17. The method of claim 14, further comprising:

2 identifying a peak of the first derivative function, wherein the fixed location on
3 the first derivative function is the peak of the first derivative function.

1 18. The method of claim 17, wherein the integrating is performed from the
2 peak on the first derivative function toward the beginning of the first derivative function to
3 obtain the respective integrated areas.

1 19. The method of claim 14, wherein determining the beginning of the
2 baseline region comprises:
3 determining whether an increase in the value of one data point to a next data point
4 is greater than a predetermined amount; and
5 if the predetermined amount is exceeded, selecting the beginning of the baseline
6 to be after the one data point.

1 20. The method of claim 14 wherein determining the beginning of the baseline
2 region comprises truncating one or more data points from the baseline region if the data points of
3 the baseline region do not approximate linear behavior within a threshold value., wherein
4 truncating one or more data points from the baseline region comprises:

5 i) defining the baseline region as the data points from the beginning of the set data
6 points to the determined end of the baseline region;

7 ii) analyzing the baseline region to determine whether the baseline region exhibits
8 non-amplifying behavior based on the linear behavior of the data points within the baseline
9 region;

10 iii) if the baseline region does not approximate linear behavior within the
11 threshold value, removing at least one leading data point from the baseline region;

12 iv) repeating steps ii-iii until a) the baseline region exhibits non-amplifying
13 behavior, or b) steps ii-iii have been repeated a predefined number of times.

1 21. A method of determining a reference value of a biological and/or chemical
2 reaction from a data set representing an amplification process of the reaction, the method
3 comprising:

4 receiving a set of data points representing a curve having a baseline portion and a
5 growth portion, each data point representing a physical quantity of a substance during an
6 amplification process;

7 determining, with a processor, a function that approximates the set of data points;

8 calculating, with the processor, at least two derivatives of the function;
9 identifying a respective time in the amplification process where each derivative
10 has a maximum value;
11 calculating the reference value of the biological and/or chemical reaction as a
12 weighted average of the respective times.

1 22. The method of claim 21 wherein the at least two derivatives include the
2 second derivative and the third derivative.

1 23. The method of claim 22, wherein the second derivative is weighted by (1-
2 p) and the third derivative is weighted by p, and wherein p has a value in the range of 0.3-0.7.

1 24. The method of claim 21, further comprising:
2 setting the weights for the weighted average such that the efficiency of a reference
3 curve is 100%.

WHAT IS CLAIMED IS:

1. A method of determining one or more properties of a biological and/or chemical reaction from a data set representing an amplification process of the reaction, the method comprising:

receiving a set of data points representing a curve having a baseline portion and a growth portion, each data point representing a physical quantity of a substance during an amplification process;

calculating, with a processor, a first function that approximates the set of data points, wherein calculating the first function includes determining a plurality of parameters that define the first function;

extracting one or more of the parameters from the first function;

using the one or more extracted parameters to calculate, with the processor, a second function that approximates the set of data points, the second function having a different functional form than the first function; and

determining, with the processor, one or more properties of the biological and/or chemical reaction using the second function.

2. The method of claim 1, wherein the one or more properties include a quantitation value associated with the amplification process.

3. The method of claim 1, wherein the one or more parameters are used as initial values for the second function, and wherein calculating the second function includes changing one or more the initial values to reduce an error between the second function and the set of data points.

4. The method of claim 3, wherein the second function is a modified sigmoid function that contains a drift term.

5. The method of claim 4, wherein the modified sigmoid function is represented by: $f = a_0 + \frac{1}{D} \left[a_1 + \left(\frac{a_4}{D} \right) \ln \left(\frac{D}{h} \right) \right]$, where $D = 1 + h$, $h = \exp(E)$, $E = \frac{(a_2 - x)}{a_3}$, where a_0 , a_1 , a_2 , a_3 , and a_4 are parameters determined during a fitting procedure of the modified sigmoid function to the set of data points, and where x is a cycle number of the amplification process.

6. The method of claim 5, wherein the one or more extracted parameters include a_0 , a_1 , a_2 , and a_3 .

7. The method of claim 1, wherein the one or more properties of the biological and/or chemical reaction include a time C_t in the amplification process at which the second function has a specified value.

8. The method of claim 7, further comprising:
using the time C_t to calculate a third function that approximates a portion of the set of data points by:
defining a window of time centered at C_t ;
using the data points within the time window to determine the third function such that the third function approximates the data points within the time window.

9. The method of claim 8, wherein the third function is a polynomial of sixth order or higher.

10. The method of claim 7, wherein the time C_t is determined as a weighted average of a first time where a second derivative of the second function has a maximum value and a second time where a third derivative of the second function has a maximum value.

11. The method of claim 1 further comprising:

selecting an end of the baseline portion as a first time of the amplification process, wherein an integrated area of a first derivative function of the first function from the first time to a peak of the first derivative function is within a range of specified values.

12. The method of claim 1 further comprising:
analyzing the first function to determine whether a slope of the first function exceeds a maximum amplification slope; and
if the first function does have locations where the maximum amplification slope is exceeded, then treating segments containing the locations as a non-amplifying segment of the amplification curve.

13. The method of claim 12 wherein the maximum amplification slope is $\ln(2.0)$ multiplied by a value of a data point at a location where the slope is being calculated.

14. A method of determining a baseline region for an amplification curve resulting from an amplification process of a biological and/or chemical reaction, the method comprising:

receiving a set of data points representing a curve having a baseline portion and a growth portion, each data point representing a physical quantity of a substance during an amplification process;

determining, with a processor, a function that approximates the set of data points;

calculating a first derivative of the function to obtain a first derivative function;

determining, with the processor, an end of a baseline region by:

for each of a plurality of points:

integrating the first derivative function from the respective point to a fixed location of the first derivative function to obtain a respective integrated area; and

selecting a point whose integrated area is within a specified range as the end of the baseline region; and

determining a beginning of the baseline region.

15. The method of claim 14, wherein the first derivative function includes the first derivative of the function subtracted by the first derivative at an initial data point.

16. The method of claim 15, wherein the integrated area of the selected point is a predetermined percentage of the area the first derivative function from the initial point to the fixed location.

17. The method of claim 14, further comprising:
identifying a peak of the first derivative function, wherein the fixed location on the first derivative function is the peak of the first derivative function.

18. The method of claim 17, wherein the integrating is performed from the peak on the first derivative function toward the beginning of the first derivative function to obtain the respective integrated areas.

19. The method of claim 14, wherein determining the beginning of the baseline region comprises:
determining whether an increase in the value of one data point to a next data point is greater than a predetermined amount; and
if the predetermined amount is exceeded, selecting the beginning of the baseline to be after the one data point.

20. The method of claim 14 wherein determining the beginning of the baseline region comprises truncating one or more data points from the baseline region if the data points of the baseline region do not approximate linear behavior within a threshold value., wherein truncating one or more data points from the baseline region comprises:
i) defining the baseline region as the data points from the beginning of the set data points to the determined end of the baseline region;

ii) analyzing the baseline region to determine whether the baseline region exhibits non-amplifying behavior based on the linear behavior of the data points within the baseline region;

iii) if the baseline region does not approximate linear behavior within the threshold value, removing at least one leading data point from the baseline region;

iv) repeating steps ii-iii until a) the baseline region exhibits non-amplifying behavior, or b) steps ii-iii have been repeated a predefined number of times.

21. A method of determining a reference value of a biological and/or chemical reaction from a data set representing an amplification process of the reaction, the method comprising:

receiving a set of data points representing a curve having a baseline portion and a growth portion, each data point representing a physical quantity of a substance during an amplification process;

determining, with a processor, a function that approximates the set of data points;

calculating, with the processor, at least two different derivatives of the function;

identifying a respective time in the amplification process where each derivative has a maximum value, the respective times for the different derivatives being different;

calculating, with the processor, the reference value of the biological and/or chemical reaction as a weighted average of the respective times.

22. The method of claim 21 wherein the at least two derivatives include the second derivative and the third derivative.

23. The method of claim 22, wherein the second derivative is weighted by $(1-p)$ and the third derivative is weighted by p , and wherein p has a value in the range of 0.3-0.7.

24. The method of claim 21, further comprising:

setting the weights for the weighted average such that the efficiency of a reference curve is 100%.

25. A non-transitory computer readable medium storing a plurality of instructions that when executed control a computer system to determine one or more properties of a biological and/or chemical reaction from a data set representing an amplification process of the reaction, the instructions comprising the method of claim 1.

26. A non-transitory computer readable medium storing a plurality of instructions that when executed control a computer system to determine a baseline region for an amplification curve resulting from an amplification process of a biological and/or chemical reaction, the instructions comprising the method of claim 14.

27. A non-transitory computer readable medium storing a plurality of instructions that when executed control a computer system to determine a reference value of a biological and/or chemical reaction from a data set representing an amplification process of the reaction, the instructions comprising the method of claim 21.