Iterative map approach

Kinetic models of qPCR as applied to the problem of low copy numbers

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Introduction

Real-time PCR emerged as a method of choice for quantification of nucleic acids. Different detection chemistries in quantitative PCR can be classified in three groups based on kinetic description:
1. Direct amplification product detection: SYBR Green I and labeled primers
2. Hybridization probes based detection
3. Hydrolysis probes based detection (TaqMan)

Current methods of quantitative analysis are based on estimation of the initial target size by crossing point approach or by calculating position of the second derivative maximum of fluorescence . Both methods are based on two assumptions:
1. Efficiency of amplification is constant within the course of each individual reaction and in different reactions using the same sequence template
2. Intensity of fluorescent signal for all detection chemistries is equivalent to the amount of amplified target

These assumptions have no scientific justification and often lead to erroneous quantitative results.

We approach PCR as an *a priori* multiplex system , where reactions are affecting each other whether they are observed or not.

Iterative map approach

The basic idea is that template growth is described by an iterative map

$$x_{n+1} = x_n + x_n P_n F(x_n; P_n) \qquad (1)$$

where x_n is the concentration of the template, P_n is the concentration of the primers
While this is certainly correct it is useful to recognize that the growth of template depends on both the amount of template and the amount of primer, expressed as

$$x_{n+1} = x_n + x_n(1 - x_n) f(x_n) \qquad (2)$$

For simple reactions, $P_0 = 1 - x_n$ (in scaled variables), however, when there are competing reactions this will not be the case.

Competition model

This model is easy to extend to the case where there is competition for primer between template and non-specific products: "primer-dimers". First, we assume that we know (or can determine) the growth curves for template alone and for primer-dimer alone. This gives two different growth functions, say $F_t(x; P)$ and $F_p(x; P)$. Suppose that the template population is represented by x and the primer-dimer population is represented by y. We can adapt these models to incorporate competition. We can take

$$x_{n+1} = x_n + x_n(1 - x_n - y_n)F_t(x_n; 1 - x_n - y_n); \qquad (3)$$
$$y_{n+1} = y_n + y_n(1 - x_n - y_n)F_p(y_n; 1 - x_n - y_n); \qquad (4)$$

This model forms the basis for the current algorithm for quantification analysis

Kinetic models

Analytical approximations of growth functions may be derived by solving systems of kinetic equations for each group of detection chemistry.
Although there are no closed form solutions in general, since rate constants are functions of temperature, some useful initial guesses may be derived for the growth functions. Important differences versus current quantifications approaches are:

1. Interpretation of fluorescence signal in terms of the amplified template concentration according to kinetic mechanism
2. Global curve analysis as opposed to local (threshold cycle) analysis

3. Instead of asking the question :
WHAT IS THE THRESHOLD CYCLE ?
we ask the question:
WHAT IS THE INITIAL TEMPLATE CONCENTRATION?

SYBR Green -based model

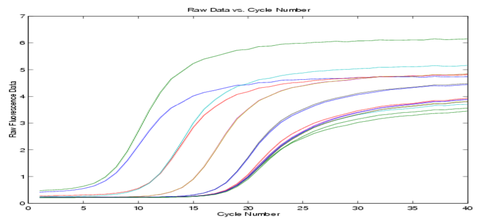
1.1 Simple Growth

We start with a simple growth law, assuming that when primer binds with ssDNA it always forms dsDNA, and the only thing prohibiting this binding is the reannealing of ssDNA. We take

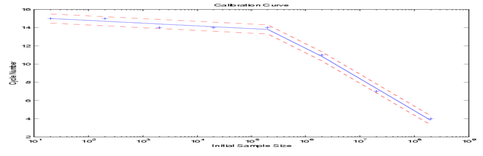
$$\frac{dT}{dt} = -k_1 PT - k_2 T^2, \qquad (1)$$
$$\frac{dP}{dt} = -k_1 PT, \qquad (2)$$
$$\frac{d\Pi}{dt} = k_1 PT + k_2 T^2, \qquad (3)$$

where T is ssDNA, P is primer, and Π is product (dsDNA). Here reannealing is at a rate proportional to T^2, which is valid if the replication of template is symmetric (i.e. $T = T^*$, where T^* is the complementary ssDNA strand).

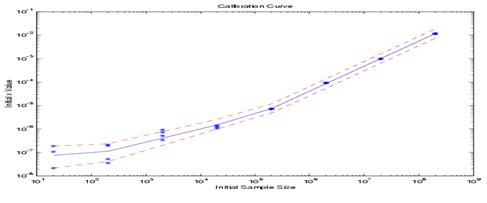
SYBR Green I amplification: dilution series



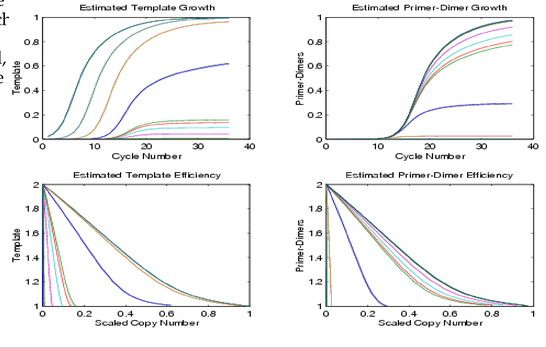
SYBR Green I amplification: dilution series. Calibration curve 2nd derivative max. method



SYBR Green I amplification: dilution series
Calibration curve (kinetic analysis)



SYBR Green I amplification: dilution series
Efficiency estimates (kinetic analysis)



Hybridization probes kinetic model

1.2 Competing Probes

Now suppose there is an additional reaction with a probe, and that binding of ssDNA with probe precludes other reactions. Then

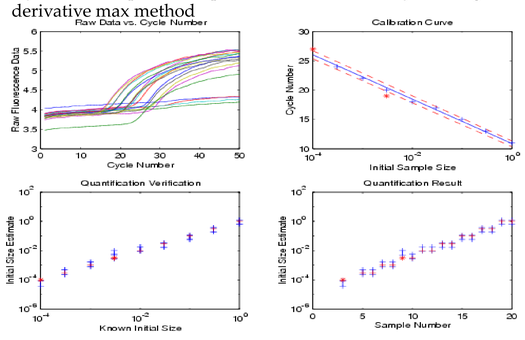
$$\frac{dT}{dt} = -k_1 PT - k_2 T^2 - k_3 RT, \qquad (12)$$
$$\frac{dP}{dt} = -k_1 PT, \qquad (13)$$
$$\frac{dR}{dt} = -k_3 RT, \qquad (14)$$
$$\frac{d\Pi}{dt} = k_1 PT + k_2 T^2, \qquad (15)$$

where R is probe. Furthermore, fluorescent signal is directly proportional to the amount of bound probe, so signal (S) is

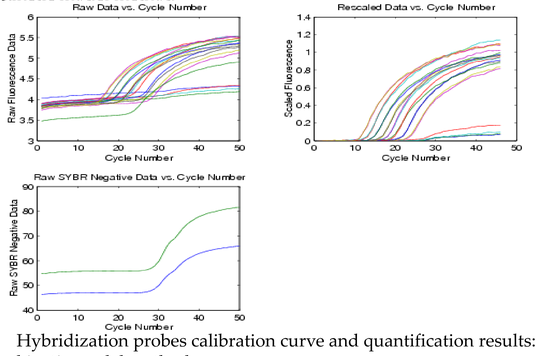
$$\frac{dS}{dt} = k_3 RT \qquad (16)$$

so that $S + R = R_0$.

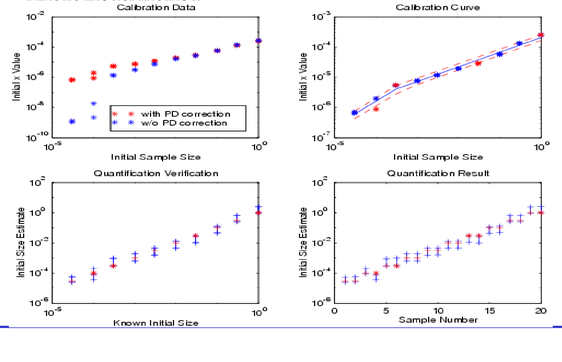
Hybridization probes amplification data and analysis using 2nd derivative max method



Hybridization probes amplification data and analysis using kinetic model method



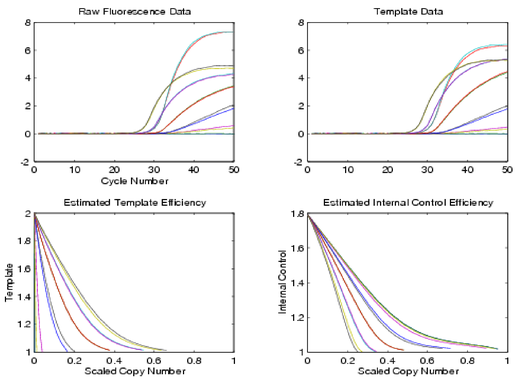
Hybridization probes calibration curve and quantification results: kinetic model method



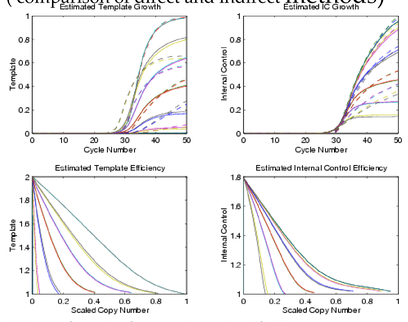
Quantifications with internal control

Similar approach can be applied to multiplex competitive reactions, where specific amplification of competitive template (internal control) affects amplification of the target template. We can either reconstitute competitive control amplification based on the target growth (direct method), or
reconstitute target amplification by analyzing competitive control growth for each sample.

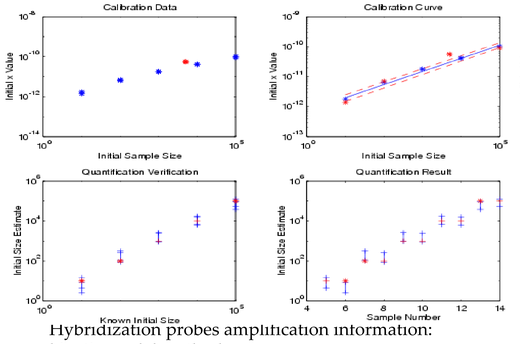
Internal control experiment with Taqman probes: target amplification and analysis information (direct method)



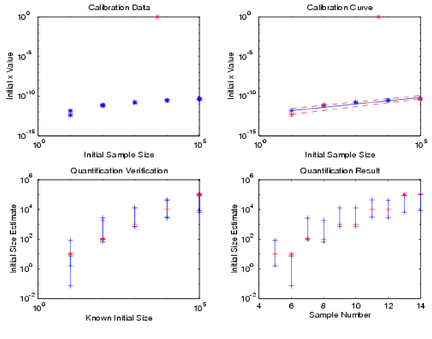
Internal control experiment with Taman probes: target amplification and analysis information
(comparison of direct and indirect methods)



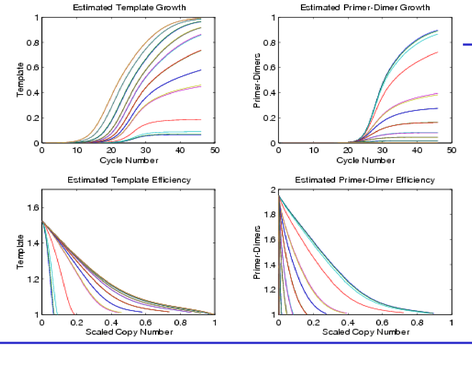
Internal control experiment with Taqman probes: calibration curve and quantification results (direct method)



Internal control experiment with Taqman probes: calibration curve and quantifications results (indirect method)



Hybridization probes amplification information: kinetic model method



Conclusion

Proposed method allows to:
Estimate changes in amplification efficiency during the course of reaction as a function of initial target concentration
Expand the dynamic range of quantifications and improve its stability
Kinetic method is also applicable to analyzing multiplex experiments

Acknowledgements

Experimental data were provided by Alpha Helix AB, Uppsala Sweden