Proceedings of the 5th International Conference on Nanotechnology: Fundamentals and Applications
Prague, Czech Republic, August 11-13, 2014
Paper No. 128

Imitational Model of Real-time Polymerase Chain Reaction with Double-stranded Template

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Keywords: Quantitative PCR, Mathematical modelling.

Extended Abstract

Polymerase chain reaction has become an important technology in microbiology, molecular biology and related fields. Despite PCR is still often used for qualitative analysis, it is normally applied as a quantitative assay in real-time format (qPCR). Theoretically, qPCR can be applied for the analysis of minimal amounts of template DNA down to single molecules which brings the method to the domain of nanoscale assay. However, the reaction behaviour at such resolution level may depend on a number of factors which have to be taken into account for correct interpretation of results. Development of effective data processing software for qPCR devices requires accurate mathematical description of the process. Numerous models were suggested to interpret quantitative PCR data; nonetheless no model can give precise description of all phases of reaction for a wide range of initial target concentrations.

When PCR is used in practice it is usually difficult to reach equal efficiency of synthesis of two complementary DNA strands. This is caused, in particular, by the differences in the temperature dependence of primer hybridization, mutations, nucleotide composition, and secondary structure of amplified fragments. This effect becomes crucial in some applications like allele-specific PCR when the reaction efficiency should be maximal for one primer and close to zero for the other one. To estimate it we have developed an imitational stochastic real-time PCR model which considers amplified DNA fragment as a complex consisting of two strands with different amplification efficiency. The model made possible to estimate the influence of different reaction parameters on qPCR results using large data sets inaccessible for laboratory experiment (thousands of repeats). To implement such scheme realistically one also has to take into account different efficiencies of synthesis from initial template and amplified copies. We introduced this distinction into the model.

Statistical analysis of model behaviour demonstrated clear difference between two situations: 1) inefficient priming with the initial template and efficient priming with the amplicon; 2) inefficient priming all over reaction. The first situation takes place when primer design was successful but template contains mismatching sequence due to mutations which can be intended in the case of allele-specific PCR or undesired in all other cases. In this situation, the reaction will demonstrate a constant delay in some cycles compared to perfectly matching template whereas its efficiency and accuracy will be normal allowing analysis of single DNA molecules. The second situation takes place when primer design was erroneous per se and it doesn't match reaction temperature or the amplicon sequence contains secondary structure interfering with primer hybridization or extension. In this case total PCR efficiency observed in sample dilution experiments will be reduced. Quantification accuracy will dramatically decrease for small

number of initial copies. However, for large number of copies (above some hundreds), stochastic variation due to low reaction efficiency will be dominated by random fluctuation of initial copy number resulting from sample pipetting.

Our modelling approach can be used for further studies of qPCR statistics and for developing novel algorithms of data processing in real-time PCR software.