

## • virtualPCR

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### 1. Introduction

*This readme gives a short introduction to the program and a brief view on the theory behind the program. When real-time PCR is familiar to you, you can skip this part. If not, reading the article on the URL below can be clarifying for the rest of the document.*

Polymerase Chain Reaction (PCR) is a commonly used scientific method to amplify specific sequences of a pool of (c)DNA and to quantify those sequences. A PCR reaction is a cyclic reaction where the reaction-product (amplicons) are visualized on a gel. The modern variant of PCR is real-time PCR. In contrast to the classic technique the latter is a dynamic detection technique, hence the name, and not an endpoint detection. This methodology has some advantages, the increased accuracy being the most important. More information on real-time PCR can be found on the following URL:

- [http://en.wikipedia.org/wiki/Real-time\\_polymerase\\_chain\\_reaction](http://en.wikipedia.org/wiki/Real-time_polymerase_chain_reaction)

This program is developed as a model for the real-time PCR reaction. It was intended to have an educational value but recently it can also be used as a research tool.

### 2. The theory...

So the reaction starts with a mix of subject cDNA, primers/probes, dNTPs, and DNA polymerase enzymes. Every cycle the DNA region of interest is duplicated by the polymerase enzymes. This duplication is actually the main theme of this real-time PCR reaction, resulting in the following equation:

$$x_n = x_0 2^n \quad [1]$$

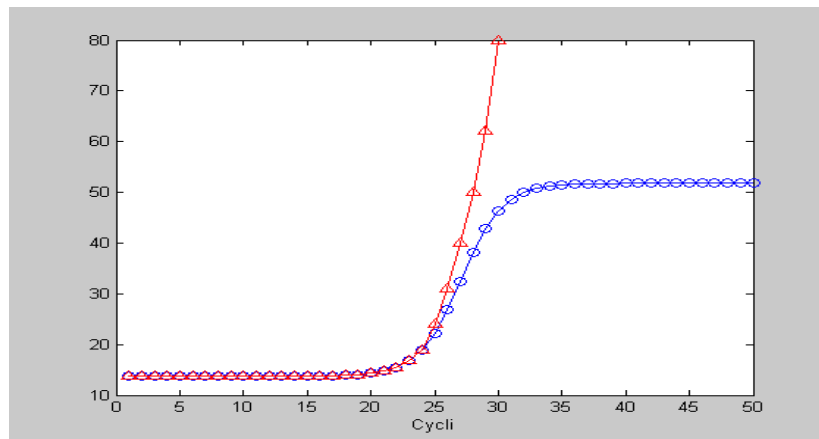
Where  $x_n$ , the reactionproduct at cycle n, is equal to the product of the initial amount of cDNA,  $x_0$ , and 2 to the power of the number of cycles.

$$x_n = x_0 E^n \quad [2]$$

Equation (EQ) [1] assumes that the efficiency, of this reaction (mainly determined by the quality/sequence of the primers and probes), is equal to 2 (=100%), which is theory. So we rewrite this equation: EQ [2].

$$R_n = R_0 E^n \quad [3]$$

The amount is never detected. Since real-time PCR is based on fluorescence, this emitted light is measured per cycle as surrogate marker for the amount of amplified material. Our EQ [3] results in the red curve on the figure below.



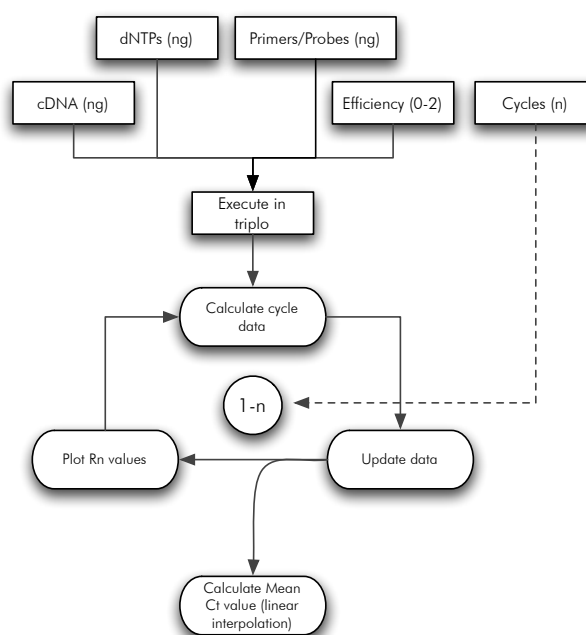
In reality, the amplification curve shows a sigmoidal track, and follows only partially the exponential curve. This sigmoidal curve can be split in 3 phases:

- Initial phase: the amplified material results in a fluorescence below the detection limit.
- Exponential phase: a steep rise in fluorescence, the theoretical function and the real function correspond in this phase.
- Plateau phase: after a while, the dNTPs or Primers/Probes are depleted and there's a decline of the efficiency of the reaction. The reaction stops when the efficiency is equal to 0.

Logically the exponential phase, where EQ [3] can be applied, is the most interesting phase. It is in this region that a Ct-value is determined. This means that, in the exponential phase, a threshold value for the fluorescent signal is defined. The Ct-value of an amplification reaction is the point in time (cycles) where the detected fluorescence crosses the threshold. These Ct-values are then used to compare expression patterns between different amplified genes/samples.

This algorithm is a model of a real-time PCR reaction, not following the theoretical model but the real-model. The efficiency of the reaction, the number of cycles, the amount of dNTPs, primers/probes and template cDNA are taken into account.

The flowchart below illustrates the structure of the algorithm:



### 3. Installation & usage

Installation is very simple. Unzip the file to a preferred location. Open Matlab and navigate to the unzipped folder *virtualPCR*. Type *virtualPCR* in the command window and hit enter. A little interface opens. To add a sample just fill in values into the text-boxes and then click the *Add Sample* button. Add as many samples as you want. To run a virtual PCR, click on *Calculate*. The detailed results of the algorithm are displayed in the command window. In the interface you can see the amplification plots of the reactions. The program asks you if you want to save an image of these plots or not.

If you want to export the results by typing *global vSamples;* into the command window. Then you can see the variable *vSamples* in the workspace window. Double-click this variable to open the Array Editor. Copy, paste, etc. freely to other programs...

Clear results before running some other samples: press the *Clear Samples* button.

### 4. Dig the code

This is not a huge program, so the code can easily be read. Furthermore, to increase readability, the code is thoroughly commented. The calculations are done by the *doRTReaction.m* file.