COMPUTER EXERCISE 3: REAL-TIME QPCR DATA ANALYSIS

Introduction

The real-time qPCR machine will make amplification plots based on the intensity of fluorescence from the SYBR Green from each sample as the amount of PCR product increases with cycle number. The amount of DNA present in a sample is inversely proportional to the cycle number at which it starts to amplify. Therefore, the greater the amount of DNA in your starting sample, the earlier it will start to amplify within the reaction (Figure 1).

The Ct (cycle threshold) is defined as the number of PCR cycles required for the fluorescent signal generated for a specific sample to cross the threshold which exceeds background level (Figure 2). Ct values of <29 are positive reactions indicative of abundant target nucleic acid in the sample. Ct values of >29 indicates small amounts of nucleic acid in the sample.

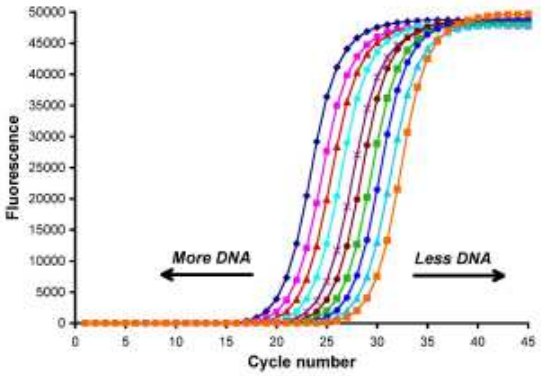


Figure 1. Amplification plots in a real-time qPCR.

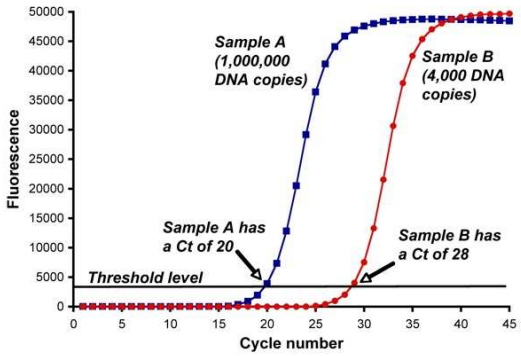


Figure 2. Ct values in a real-time qPCR (example only)

Standard Curve

Data has been generated for you using increasing known concetrations of control cDNA. The same experimental conditions as your own qPCR analysis were utilised for this purpose.

You will use this data to produce your own standard curve and from this calculate virus copy number in the two blood cDNA samples and the positive control.

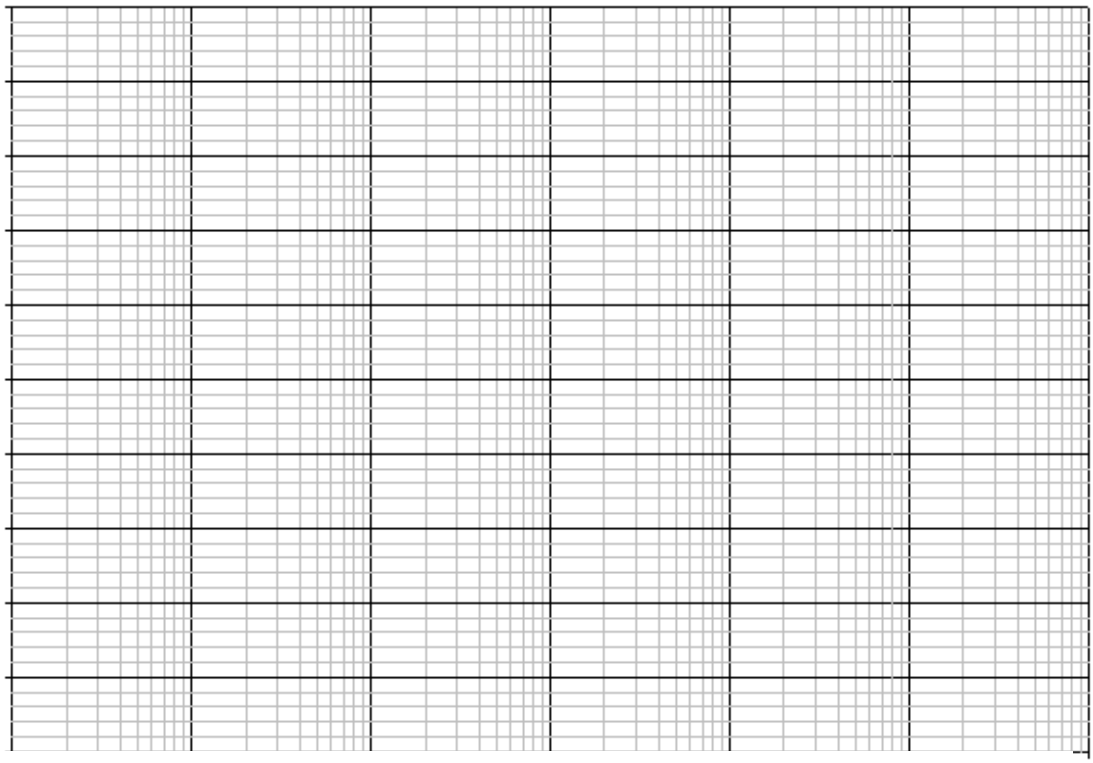
Questions

Q1. Open the Standard Curve Excel file on Moodle, and fill in the appropriate values for the Standard Curve in Table 1.

Use Excel, a calculator or the equation below to calculate Ct mean and Ct SD

Sample number	Sample (copies/ml)	Ct Values	Ct Mean
A1	1,000,000		
A2	1,000,000		
A3	1,000,000		
B1	100,000		
B2	100,000		
B3	100,000		
C1	10,000		
C2	10,000		
C3	10,000		
D1	1000		
D2	1000		
D3	1000		
E1	100		
E2	100		
E3	100		

Q2. Draw a line of best fit using the data obtained in Table 1 on the log paper provided [concentration of standard, x-axis; Ct mean, y-axis].



Experimental Data
Your data is on the Molecular Methods Moodle site. Once you have retrieved your real-time qPCR Ct values:

- Note your own Ct values in Table 2 for the reaction that you set up.
- Examine the class data and use as if they are experimental replicates.

Q3. Calculate the mean and standard deviation of the class data and fill in Table 2 (use the functions in Excel to do this).

Q4. Plot the Ct values onto your standard curve and calculate the viral load of your unknown patient samples (copies/ml) using the class data.

Sample name	Own expt Ct	Class Ct Mean	Class Ct SD	Copies/ml (class Ct)
Blood cDNA sample 1				
Blood cDNA sample 2				
Positive control cDNA				
Negative control (water)				

Table 2 Real-time qPCR data from Blood cDNA samples

Q5. Look at the Ct values of the samples that you set up. Does your data correlate with the rest of the class? If not, how can you explain the error?

Q6. Look at the calculated Standard Deviation of the class data. What does this tell you about the class data?

Q7. Can you come to a conclusion as to which patient sample has HIV?

Q8. Looking at the table below, what information could you give the individuals who had this test?

Viral load (copies/ml)	Descriptor	Notes
~100,000	High viral load: antiviral treatment recommended	
~10,000	Low viral load: further investigation required in order to determine treatment option, eg $CD4^+$ count/clinical presentation	
<50	Undetectable	Current tests can only reliably detect >50 copies/ml

Compare your results from your PCR and real-time qPCR experiments. Do these results correlate? If not, what do you think has happened? What additional information has the real-time qPCR expt provided?