Data Analysis

Homework 5

1. Look up on the course site which machine (IQ5 or CFX) was used to run your plate and analyse your data using the corresponding software

2. Pick a data file from one of your colleagues who used a different machine and analyse his/her data using the corresponding software

you are most welcome to work in pairs or in groups to analyse each others data

Absolute qPCR

1. Use software to build a Standard curve

2. Use software to find concentration of the sample B

3. Assess how good are primer efficiency and R squared

4. Check your NTC control

5. Check your Melt curve, are your results reliable?

6. (only if you feel like doing it) make manual calculations of the standard curve and the sample B concentration

7. (only if you feel like doing it) the plasmid you were using is 22kb long, convert concentration of the sample B into plasmid copies

Relative qPCR

1. Use software to build Standard curves for Efficiencies

2. Assess how good are primer efficiencies and R squared values

3. Check your Melt curve, are your results reliable?

4. Estimate fold difference of GOI, Ref1, Ref2, Ref3 in sample 1 and 2 (ΔCt test)

5. Run a $\Delta\Delta$Ct test using Livak and Pfaffl methods and compare the results

6. Find out the M values and select the most stable pair of reference genes

7. Run a $\Delta\Delta$Ct test using Pfaffl method and a pair of the best reference genes, compare this result with the results obtained using all three reference genes