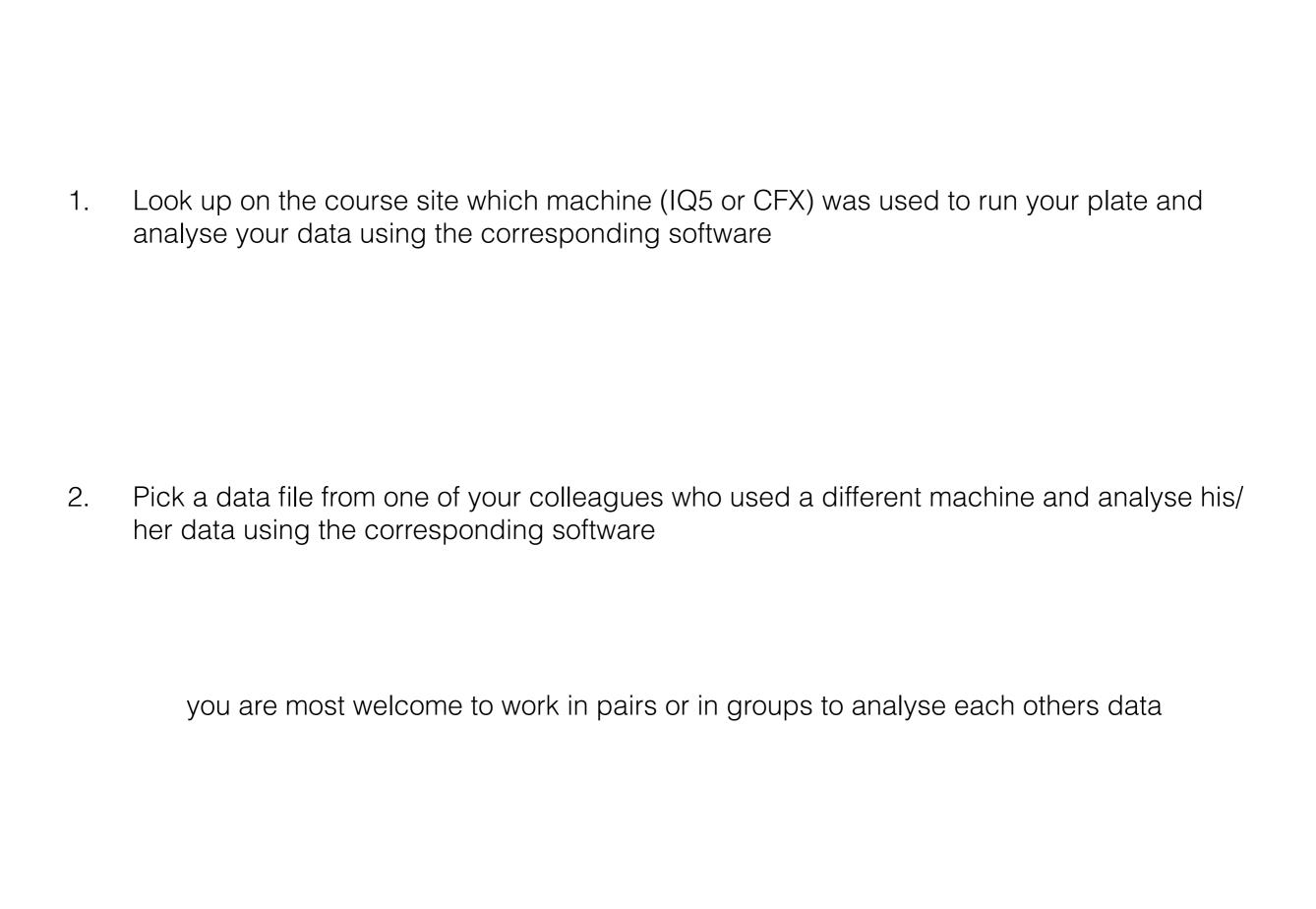
## Data Analysis

Homework 5



## 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 ΔΔCt test using Pfaffl method and a pair of the best reference genes, compare this result with the results obtained using all three reference genes