Appendix B

Subject questionnaire

Estimated Time : 20 Minutes

Tell me about your experience with qPCR experiments.

Question notes:

- RNA or DNA qPCR ?
- primers only or probe sets?
- How many qPCR experiments in last year/two?
- How many planned in next 6 months?
- How difficult would it be to reanalyse/repeat your own qPCR experiment?

Describe how qPCR experiments are used/presented in published papers related to your research.

Question notes:

- Recount a time where you questioned results/conclusion from qPCR experments
- Did you attempt to reanalyse/recreate their qPCR data?

Describe the design of your most recent qPCR experiment

Question notes:

- Technical/biological/experimental replicates?
- Plate design?
- Software to design plate (excel?)
- Methodology for ordering samples
- Number of wells?
- Typical number of probes?
- Primer efficiency calculation?
- MIQE best practises qPCR guidelines?
- How did you load your plates - single-channel pipette, multichannel pipette, electronic or manual, automatic loading with what robot?
- What qPCR instrument did you use?
- How do you tell if your experiment worked - what do you do for quality control?

Describe the analysis pipeline of your most recent RT-qPCR experiment

Question notes:

- GUI / Terminal / R based?
- Proprietary software?
- See, understand and repeat every step?
- Customisable, paper ready output graph?
- Whats the biggest frustration? (is there something you know you should be doing but don’t)

- Would it be easy to redo an experiment (because something went wrong) using the same analysis?
- The features you require from qPCR software

What is your previous R programming / terminal experience?

Question notes:

- Previous courses?
- Previous obstacles?
- Familiar with the concept of tidy data?
- Interest in learning?

Tidyqpcr worksheet

Estimated Time : 40 minutes

Follow installation instructions on https://github.com/ewallace/tidyqpcr

Read through the vignette on plate designing

Create a example plate design for the following experiment:

8 by 12 well plate
Three Biological Replicates
Three Technical Replicates + “-RT” control
One strain: “WT”
Two conditions: + and - “menadione”
Four probes: "PGK1","ALG9", " HHT2", "HTB2"

Read through the instructions on conducting qPCR analysis with tidyqpcr in the multifactor vignette

Load in the example plate plan using data(tidyqpcr_plateplan) and associated experimental data.

Normalise raw data and produce plot of differential expression under two stresses.