eLowQuant Video Outline

Welcome to the eLowQuant video!

Brought to you by Dr. Mary Lesperance, Professor of Mathematics and Statistics, and Dr. Caren Helbing, Professor of Biochemistry and Microbiology at the University of Victoria in beautiful British Columbia Canada.

The purpose of the video is to help you get set up and using eLowQuant R script to generate limits of detection (LOD) and quantification (LOQ) and associated confidence intervals for an eDNA assay based on targeted qPCR. The code generates maximum likelihood estimates for binomial-Poisson models and enables you to estimate copy number with standard error from an unknown sample when the frequency of detection is <100% in 'n' number of technical replicates.

We will cover how to set up RStudio, introduce you to R Markdown, and then run through an example of how to use the eLowQuant script.

Let's get started!

1) **RStudio – get latest version**
    a) Set up console windows
    b) Global options: Pane Layout – set up panes; R Markdown – turn off Show output inline

Let's have a quick review of the R Markdown features that we will be using.

2) **R Markdown – create small sample file - File>New File>R Markdown**
    a) Review parts of the file
    b) How to run chunks - see the output in the Console

eLowQuant can generate three types of reports through the process of knitting. From experience, many people get tangled up in this process, so it is worthwhile to show you how to do this with a simple example.

    c) Knit to html (knitting can get you in stitches)
    d) Knit to Word
    e) Knit to pdf (tinytex may be needed, see later)

Now let's set up the RStudio environment for eLowQuant.

3) **Set up for eLowQuant example**
    a) Create a new project using a new folder called eLowQuant: File>New Project
    b) Within the new project folder create a folder called Outputs
    c) Put other files needed in the main project folder: EXAMPLE-eLowQuant.csv, eLowQuant-V20210813.Rmd, eLowQuant-Functions-V20210407.R
    d) The script requires a specific input file format (csv) with a specific set up. (Open up EXAMPLE-eLowQuant.csv file in Excel to point out columns, for non-detects, set Cq to be empty or NA value, include negative controls, do not duplicate target names over different labs)

The moment that you have been waiting for …. open and run the eLowQuant script.

4) Open eLowQuant.Rmd

a) Review the instructions. Show where the file name of the data resides in script and indicate that this name can be changed according to your needs.
b) Run the chunks. Some produce data on the console and some do not.
c) Pay close attention to the output as it is being produced. Make sure that it makes sense and that there are no errors (Warnings are OK).
d) Graphs are in the "Plots" window. Note that for the last set of plots generated in the last chunk called "Manuscript", the y axes of all plots are set to the same maximum to facilitate comparisons.
e) The output from the "Manuscript" chunk will indicate which model (no intercept or intercept) is the best for each eDNA assay. Use this information to select which Sq estimates you will use. Another way is to look at the likelihood ratio p values. The largest p value is the best fit.
f) Sq estimation can be adjusted to the desired number of technical replicates by modifying the appropriate code in the "MLES0fits0vec" chunk for the no intercept model or the "MLES0fitsvec" chunk for the intercept model.

We hope this video was helpful.

Have fun with eLowQuant!

Resources

1. eLowQuant R code available here: https://github.com/mlespera/eLowQuant
2. Lesperance, M., Allison, M.J., Bergman, L.C., Hocking, M.D., and Helbing, C.C. 2021. A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 00: 1-12. doi: 10.1002/edn3.220.