Instructions for qPCR data extraction using SDS software (version 2.4)

1. Open file, highlight wells of interest
2. Scroll down sample list and look for flags and confirm blanks
3. Check QC summary (select the tab) for any issues; make notes as needed to exclude wells, etc.
4. Select the "Results" tab and you'll see a plot (labeled "Amplification Plot"); check Ct vs. well to look for bias across plate by selecting it for the plot (select from the dropdown menu when looking at the results tab)
5. Check defaults (again, in the results tab):
    - detector: Fam
    - plot: delta Rn vs cycle
    - -color: well
    - -y-axis: Log
    - -threshold (see below)
6. Adjust the baseline (red triangles):
    - left one typically move to 1- (triangles tell the program which cycles to ignore as it makes the calculations. We set it to 1 so that it only starts incorporating data where the amplification starts)
    - right one move to when amplification starts
7. Adjust threshold (green line) to be in the linear phase of the amplification.
    - This can be done by entering a value in the correct spot below the amplification plot if a person likes working with round numbers (also makes it easier if you need to go back and re-measure anything).
8. Can use the "lasso" tool at the top of the amplification plot to select on certain samples/figure out which curves match to which samples-especially useful for figuring out which sample has something funky going on, etc. Also there's the zoom (plus sign) and select (hand) for investigating manipulating, etc. (e.g. zooming in to make sure you put the threshold through the linear phase, etc.)
9. You can do this individually by gene (column) if needed, but note that it changes back for ALL the wells if you highlight another column and move it-so if you are doing it gene by gene, you need to export the data for each one before moving on to the next.
10. ALSO, if you have multiple plates, the gene for ALL plates for samples you are comparing need to be set at the same conditions (baseline and threshold)
11. Once you have things the way you want, click "Export" on the file menu (may be able to export from multiple plates at once with "Batch Export"-need to look into)
12. Make note in separate document of strange samples to later look into/remove after export,etc.
13. Save as-will save at .txt, then can convert to excel and combine plates-continue to analysis pipeline to import sample and gene information and conduct analyses
14. If using downstream R pipeline, save all the .txt files into your project directory folder (if not, can delete these in excel or whatever you spreadsheet software you are using, but will need to be deleted before going into any stats program most likely).
15. To do this, you will need to quickly open up the files and delete the extraneous info at the top and bottom so can be read as normal data files (basically just highlight all the stuff above what would be the column headers, and then the stuff below the data rows with the slope, etc.).

- We usually save these with a different name (like LK.plate1.edited.csv) so have the original .txt file in case need to go back and check it, etc.
- See R pipeline script for further instructions as needed