ABI 7700 / SDS 1.9.1 Software Instructions **5 September 2006**

The ABI7700 has a curious "feature" where different wells on the plate are read different numbers of times during a given temperature hold. (In general the top left of the plate is read more than the bottom right.) This could potentially affect results if the top left of the plate suffers more fluorescence bleaching than the bottom right. More important, the different number of reads makes it very difficult to undertake spreadsheet analysis of the data, because the fluorescence values are not easily aligned by timepoint. The software here will align the well reads at the same time points, with one well in each column.

The first program (flip) will change the formatting of the exported file from Macintosh to Unix (changes "linefeed" to "newline"), to allow the second program (reformat) to work.

1. Move the flip and reformat programs to the home directory of a Macintosh with OS X.
2. Export a multicomponent file from an annealing run and put it in the home directory as well.
3. Open the Terminal program. The first time you use flip after downloading it you need to acquire permission to execute it by typing

    chmod u=rwx flip

    To run flip type

    ./flip –u **filename.multicomp**

    where **filename.multicomp** is the name of the multicomponent file.
4. Run reformat (also in the Terminal program):

    awk –f reformat **filename.multicomp**
5. The reformatted file will be called output.txt and it can be imported into Excel. It shows SYBR fluorescence intensity versus time for each well, normalized to the ROX passive reference.
6. A companion file called qc.txt can also be imported into Excel. It shows the actual values for SYBR, ROX, background, and mean standard error that were computed by the SDS multicomponent algorithm. This data can be useful for troubleshooting but is not necessary for routine analysis.
7. Remove the output files from the directory so that when you run the reformat program in the future, the output goes to a fresh file and is not appended to the old one.
8. Further analysis steps have not been automated:

    a. Calculate fluorescence intensity relative to a reference (unmelted) control well.

    $NF = SYBR_{sample}/SYBR_{reference}$

    b. Calculate annealing curve by subtracting the average NF intensity during the melt step from all values for that well (i.e. melt step is 0% annealed). After the values are zeroed, the annealing curve can then be plotted by considering the pre-melt baseline NF reading as 100% annealed.

    c. Change annealing vs time curves into annealing vs Cot curves by using baseline normalized fluorescence intensity as a proxy for the relative concentration for each well. Multiply (time)*(baseline SYBR) to get Cot values.