

## Protocol

### Data Analysis

The data from your q-PCR run should now be loaded onto the lab laptops as a file with a light-bulb icon and ending in .tad2. Double click on the file to open it, ignoring the warnings that your laptop can't directly communicate with the q-PCR machine itself. Everyone's samples will be available for you to examine but begin by analyzing your own.

One place to start is to examine the data for the DNA standards you've run. Find your samples in the 96-well dish shown in the upper left of the window and select the 6 wells that you included to generate your standard curve. The data related to these wells will appear in the lower left window, color coded as shown in the 96-well dish shown in the upper left of the window. By scrolling your mouse over the curves in the lower left window you can see the data for each sample, data that is generated using the program's default options. Before this data is meaningful, though, you will need to enter the values for the DNA standards.

This can be done by selecting the "Master" file from the upper left list on the left-most portion of the window. Next select "edit" from the "plate setup" portion of the window. This will allow you to specify your DNA standards. Select the 6 relevant wells then click the "specify quant standards" found on the lower right of the window. Choose "copies" from the pull-down units menu and enter the number of lacZ copies in each of the 6 standard wells. You must use the "enter" button on your computer to move from cell to cell on the spreadsheet (don't know why). When you've entered all the relevant values, select "OK" then "OK" again to return to the master data file.

Select "quantitation" and you'll be able to play with the threshold options, found in the right-most section of that window. Settings that have worked in the past include selection of "subtract blanks" as well as selection of "subtract baseline" options. See how the different minima affect the output of your DNA standards, selectable by scrolling the mouse over the wells shown in the upper left of the window. You can increase the cycle range to 35 (since we ran 35 cycles) and you can manually set the threshold to 0.07. You can try this or any other setting you prefer and examine how these affect the titration curves. You should use the analysis software to determine the equation and r-squared for your standard curve. You'll have to "select wells" and deselect the other standards from consideration. Deselected wells will appear as red-points on the data curve. Alternatively you can modify the master data file so the other blanks in the class are now "blanks." If you decide to leave off certain points, note which and why.

Next you'll want to assess the number of lacZ copies that were measured in each of your two experimental samples. This can be "eyeballed" by selecting the relevant wells from the 96-well dish shown in the upper left of the window and scrolling over the curves

shown in the lower left of the window. Do you see a difference in the plus and minus RT samples? Do the triplicates correlate well? Do you see a difference under the two conditions you measured? Do these differences agree with the b-gal data you measured on the cells? Record all this in your lab notebook and also begin to consider how you will enter it in the Registry of Standard Biological Parts.

The data from your run has been collected by the analysis software and can be seen by selecting the "quantify calculations" tab at the bottom of the window. You will have to select all 16 of your samples to see them listed and then choose "show selected wells" from the well selection menu. The data here includes the Ct and the copy number according to the standard curve you used. Export it to an excel spreadsheet by copying it to the clipboard then opening the data in Excel. You will have to copy and paste the standard curve equation and r-squared separately. You should also note which primer pair you used for your experiment since different primer pairs may behave quite differently depending on the efficiency with which they amplify and the stability of the region they anneal to. Hand in this spreadsheet before you leave lab. You may also want a copy for your lab notebook.

The copy number shown on the spreadsheet refers to the number in the q-PCR itself....not a particularly meaningful number on its own. Knowing the number of cells you used for your RNA preparation and knowing what fraction of the RNA you used for the RT reactions, then the q-PCR, you can try to convert the number to copies per cell, recording the data in your notebook and preparing it for the Registry.

Since the q-PCR results are indirect readouts of dsDNA you should examine the melting curve associated with your samples. What can this tell you about each reaction? Note your findings in your notebook as well as describing them on the Registry.

## **Jamboree-lite**

For the past few summers, teams made mostly of undergraduate students have participated in a genetically engineered machines competition. The teams then gathered in the fall for a "Jamboree" to show off their successes from the summer's work. The bacterial photography system was initially presented at such a jamboree in November 2004. To finish today's lab, you'll have a chance to share with the group what you have proposed to build in your essay. What do you think about its usefulness? Are you motivated to solve a particular problem? Are there existing designs that inspired you? Are there people who gave you some good ideas? Did you need to specify special parts for your idea? Do you know how to get them? What do you think are your chances for success? Does your design fit cleanly into your model for synthetic biology? What will be needed to make it a reality? Do you want to build it? Do you want someone else to build it?

Say what you like but be prepared to talk about your essay and to ask others about theirs.