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Color Compensation on the Roche LightCycler 2.0 Instrument using LightCycler 4.0 Software-

Selecting a subset of dyes from a CC file

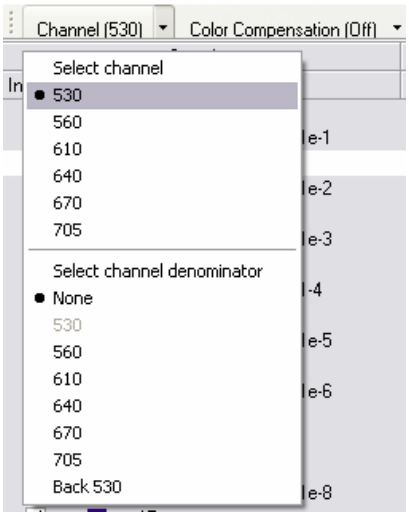
How do I use this guide?

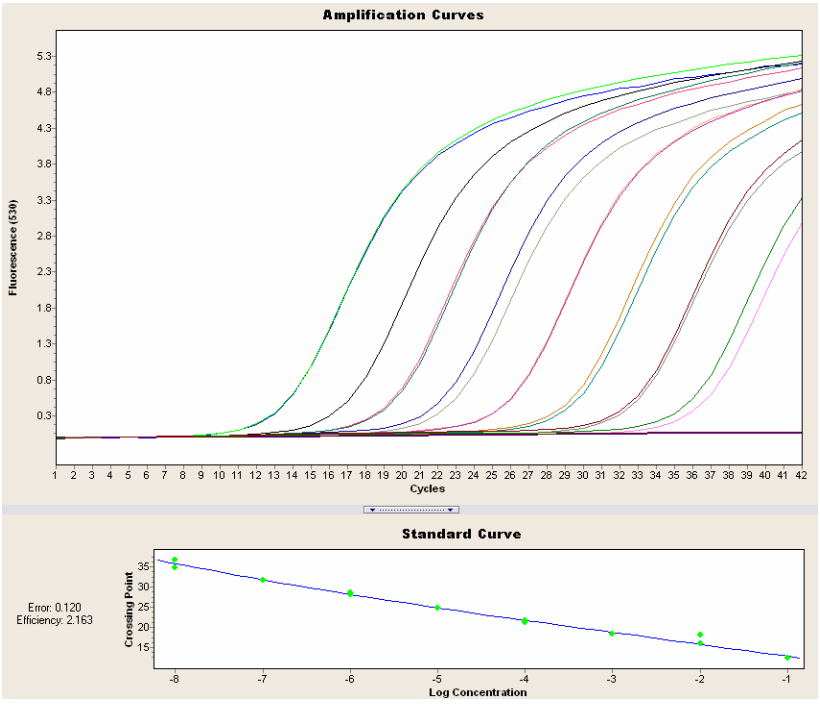
This guide is formatted in a FAQ style; however, simply following the individual questions from beginning to end will provide the step-by-step instructions to perform a Color Compensation on the LightCycler 2.0 instrument using a subset of dyes from an existing color compensation file.

What will the data look like before I color compensate for the dyes?

In the case of the FAM dye which emits across the entire spectrum that the LC 2.0 instrument can detect you will see that the fluorescent signal is detectable in each of the channels of the instrument. Here are three example channels.

Channel (530)—before color compensation:





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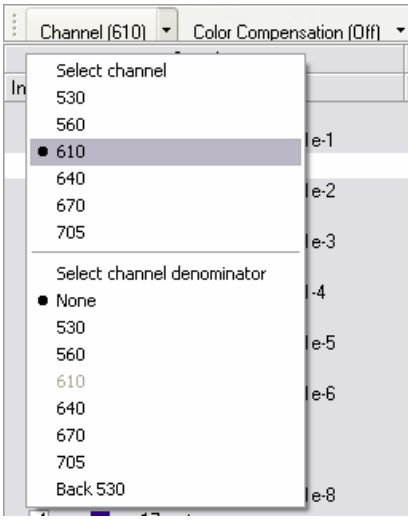
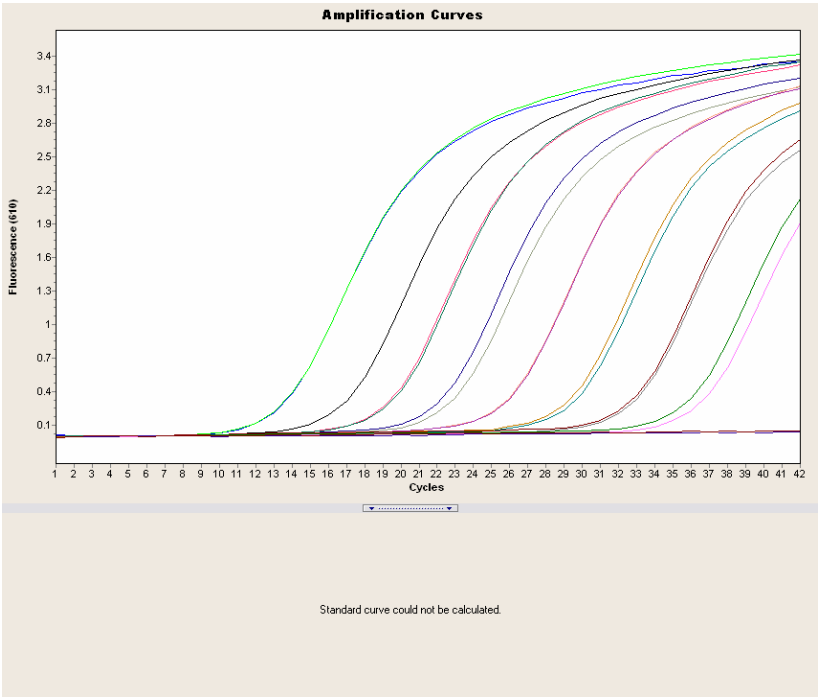
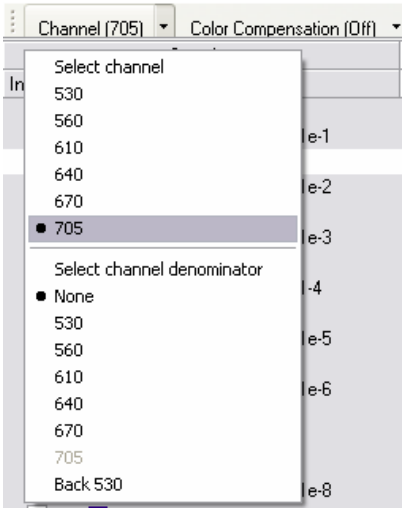
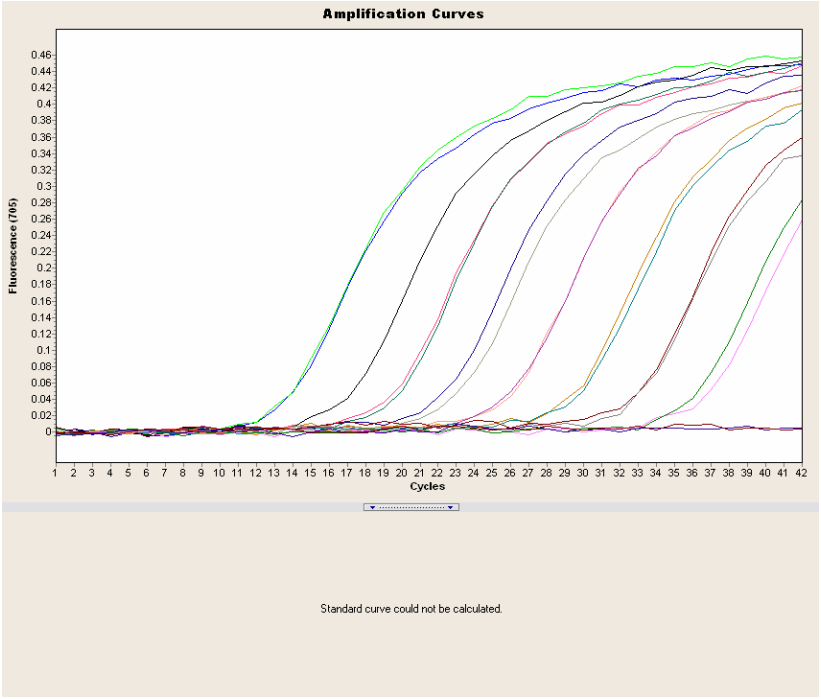
Channel (610)—before color compensation:





Channel (705)—before color compensation:





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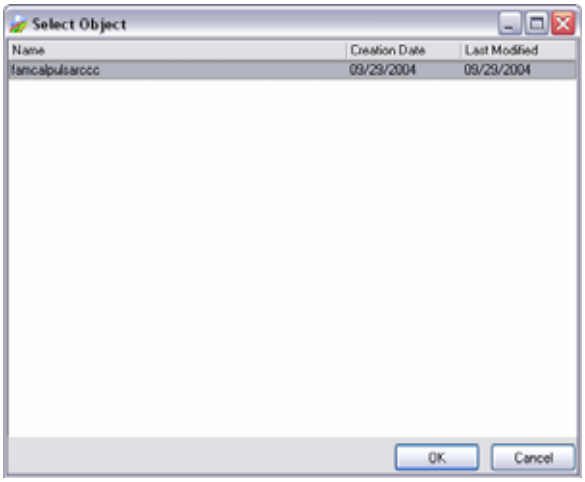
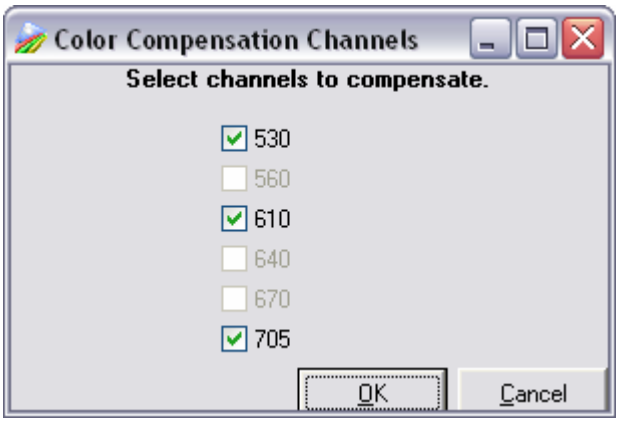
How do I use an existing color compensation file, which has more dyes in it than I need to compensate?

In this example we will discuss a trivial example of wanting to compensate with only one dye (though this is unnecessary, typically). When you first open the choice of color compensation files you will see a menu of the choice of files. Similar to the figure below:

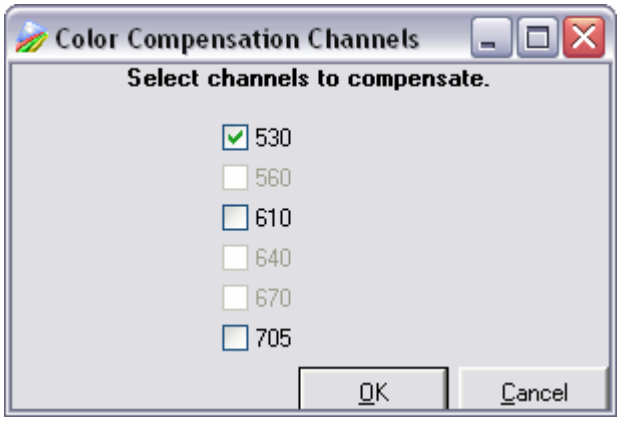


Select the file that you would like to use. It can contain more dyes than you need, but MUST contain all of the dyes you are wanting to color compensate.

Once you have selected the file and click the OK button another window will appear, showing the dyes that are compensated in the file. In this case the window looks like the figure below:



In this file, there are three dyes in the color compensation file. Each of the dyes is marked with a check. To choose the dyes that need to be compensated in the current analysis simply uncheck those dyes that will not need to be compensated. See the figure on the next page, for an example of using only the FAM dye in the 530 channel.



Here just the 530 channel dye (FAM) has been chosen. Again, this is not typically necessary to do for one dye.

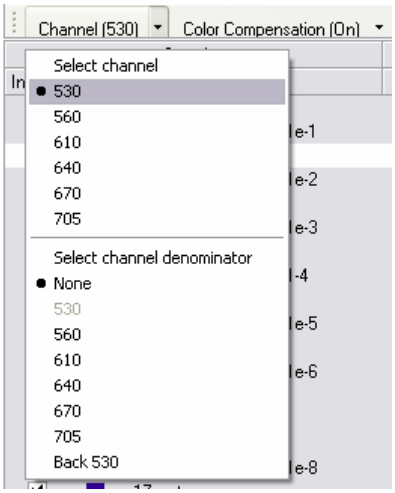
Select the OK button and the data analysis screen will re-appear. The mouse pointer will show an hourglass icon, indicating that the LC 4.0 software is performing the color compensation calculations.

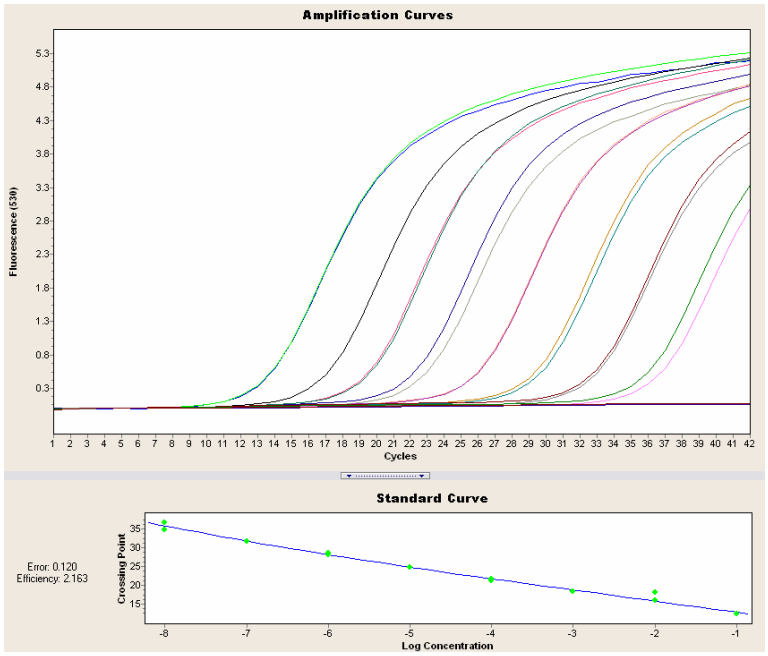
What will the data look like after color compensating?

To view the data output in each of the channels after color compensation simply use the Channel selector menu in the analysis window.

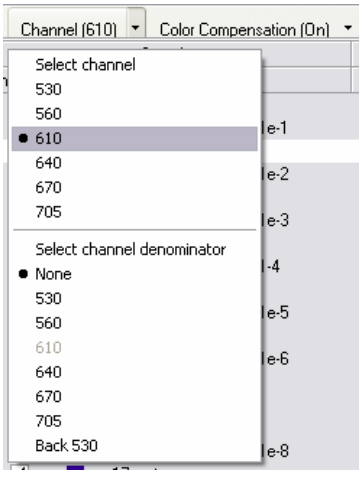
Each channel for the dyes from this run file are shown below:

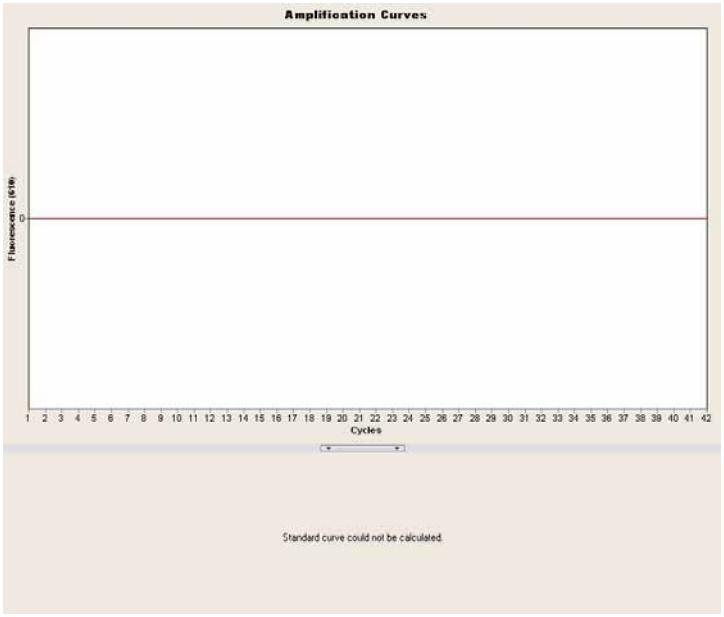
Channel (530)—after color compensation:





Channel (610)—after color compensation:





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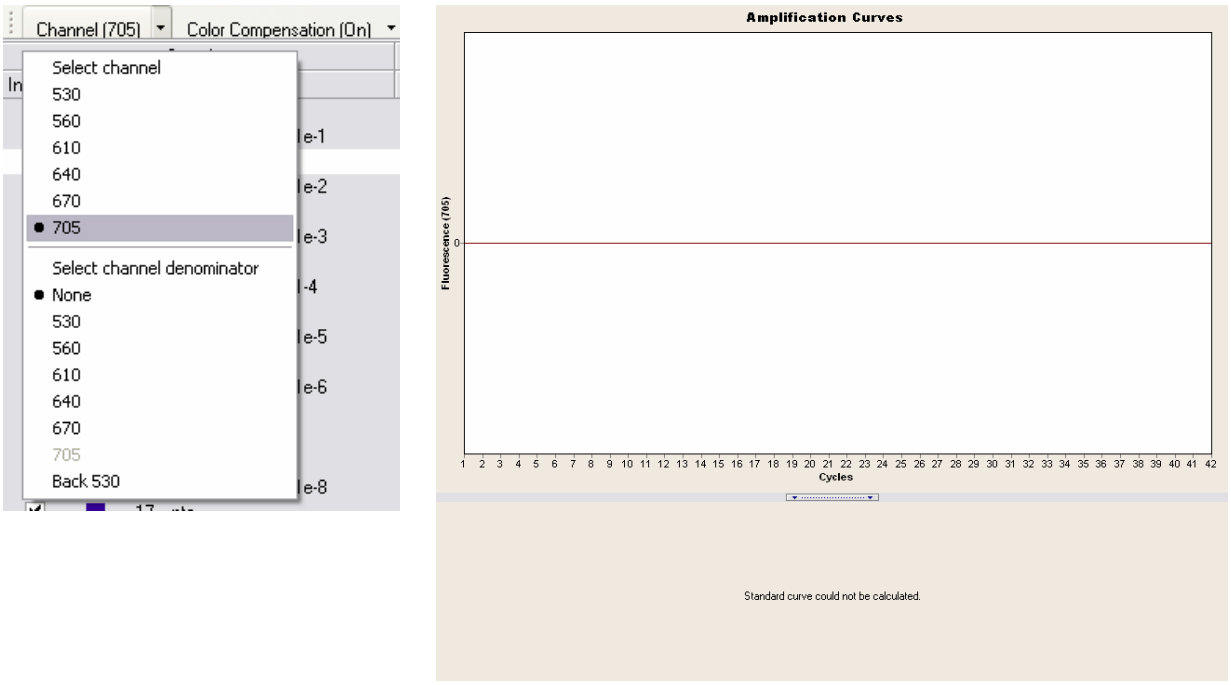
Channel (705)—after color compensation:



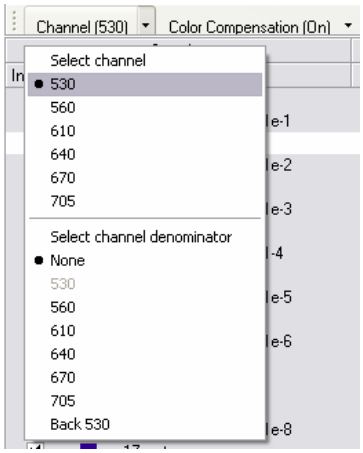
In the case of the Channel (610) and Channel (705) the appearance of a flat line at 0 indicates that the signal from the FAM dye has been properly subtracted from these channels.

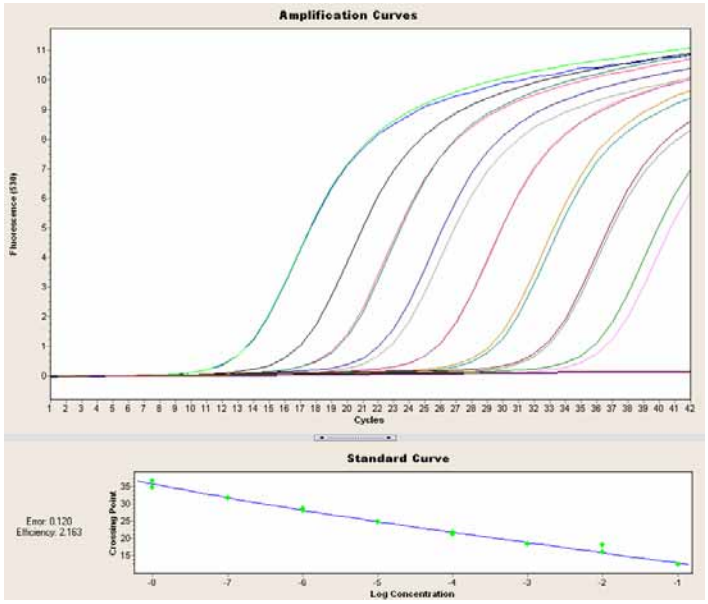
What would happen to my data if I select the wrong color compensations for my run?

Below are examples of data that has been color compensated for the 530, 610, and 705 channels of the LC 2.0 instrument with only the 530 channel producing signal.

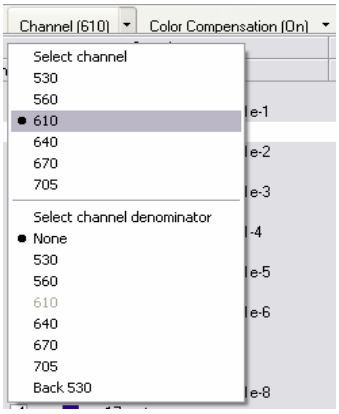
You will note that the results are varied in each of the respective channels. This is primarily a function of the software attempting to compensate for signal generation where there is actually none. Also note that the channel(s), in this case 530, is actually producing results that are similar to the properly compensated data with the sole exception being the magnitude of the fluorescence signal as seen in the y-axis of the amplification curve graph.

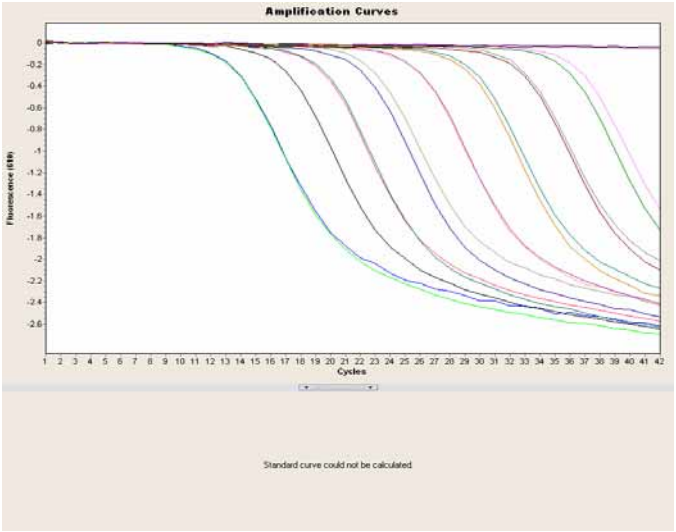
Channel (530)—with wrong color compensation!





Channel (610)—with wrong color compensation!





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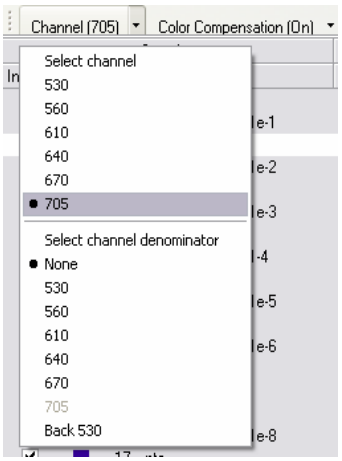
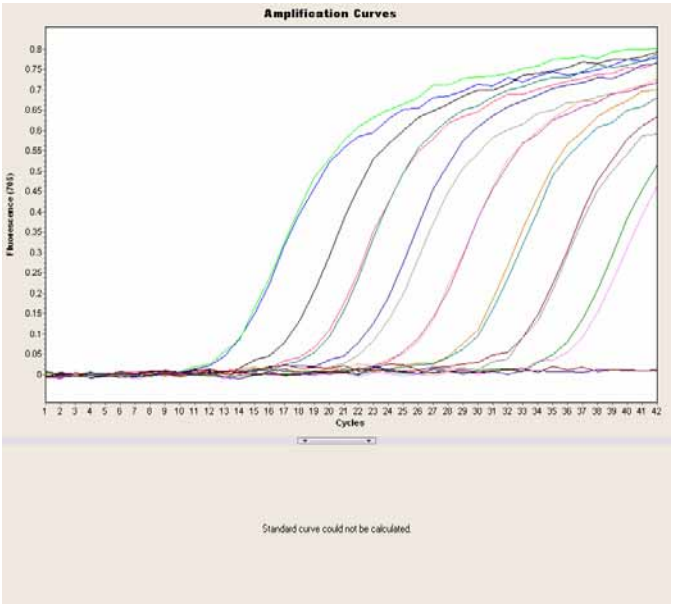
Channel (705) – with wrong color compensation!





The fundamental observation with this data set, although trivial in its application is that when you attempt a color compensation you should be sure that the color compensation file that is being used represents accurately the dyes that are being used in the run and that you have properly selected the appropriate dyes and channels to be compensated.