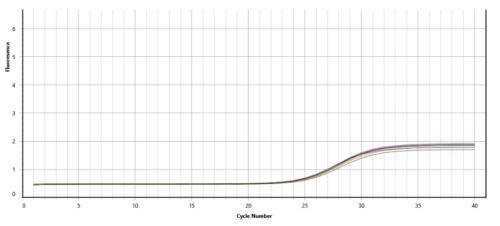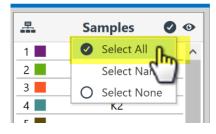# Remove Tubes from Last Run

The Problem

Let's assume you did a full rotor run and then in the next run you used just 8 samples.

You then observe that the signals are really low, as shown below. You might wonder if this is due to bad probe design, low concentration of probes in your reaction, or just poor set up. But in this case the issue is quite simple.
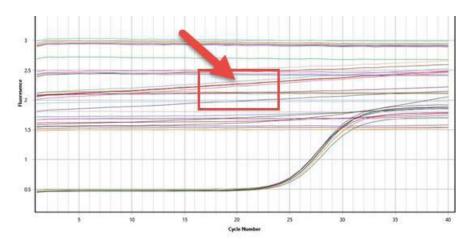
The Issue

Low signals are all too often caused by old reactions left in the rotor from the previous run.
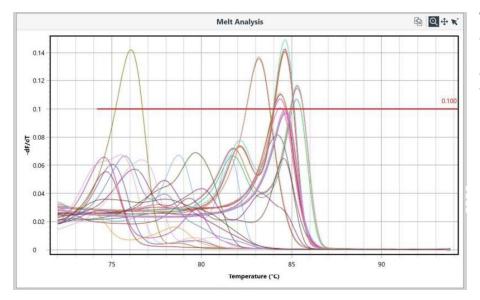

To find out, click on *Select All in the Samples selector.*

Now you can see that most of the unused reactions have a higher baseline signal. The highest signal starts at a value set for the gain adjustment (in this example it's 3 units, which is the default for intercalating dyes).

With this example, running a melt analysis, clearly shows that the higher baseline signals are indeed caused by the old reactions left in the instrument.

Why Does This Happen?

When the instrument starts, it will adjust the gain to ensure the data is within an appropriate scale for the chemistry used. By default, the instrument will adjust the gain using the tube with the highest fluorescence reading. Therefore, tubes that are left inside from a previous run, will have a higher fluorescence signal than the new reactions. So, the gain for the new reactions will be set too low.

How to Avoid This

Always take out the reactions from the previous run. It's also good laboratory practice as it reduces the chance of accidental contamination.

Ask yourself, what are the reaction volumes you normally use. Then prepare a set of water load tubes, at that volume, to use in subsequent reactions. As the tubes contain an oil overlay they can be used for over a month without any loss of volume. Store them near the instrument in the provided plastic racks. You can also prepare different volumes if required.

Alternatively, select a specific tube to run the gain adjustment on. This is done through the *Profile Editor* for the specific run (independent of the assay profile).