Thermal Shift Assay Protocol for Applied Biosystems 7500 RT-PCR

Turn on thermocycler and open 7500 System software on PC – must be done in this order so the software can synch with the thermocycler. Open a new project.

Plate Setup
1. Keep default settings on first window (assay type: absolute quantification, plate size: 96 well, etc). Click Next to move to next window.
2. Choose dye for reaction – choose "Thermal Shift – TAMRA" and click on "Add" to select dye. Go to the Passive Reference box and change it from ROX to "none". Click Next to move to next window.
3. Highlight wells to be read on the plate model on the bottom of the screen (should be highlighted in black). Click the box next to Thermal Shift on the top – each highlighted well should now have a "U". Click Finish to move to the next step.
4. Now you should see a full screen of the plate setup (see tabs for Setup, Instrument, and Results above). If you forgot to turn on the thermocycler before initiating the software, it will give you an error message that the thermocycler is disconnected. You must close the program, start the thermocycler, and repeat the above steps.

Instrument Setup
1. Click on Instrument tab to modify thermocycler protocol. Click on second stage until it has a black background and delete it.
2. Adjust temperatures and cycle number as indicated --------------------→
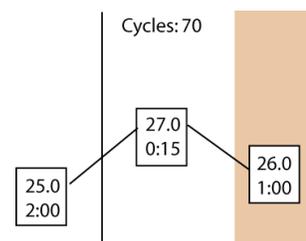
3. Go to the Auto Increment tab and enter a value of 1.0 in the temperature box (top box) for each of the two boxes in the second stage.
4. Change the reaction volume (bottom of page) from 50 to 100 ul. Brown tab (above) indicates that plate will be read during this time – this is also stated explicitly below the reaction volume box.
5. Save file in Gardner lab folder in Users.
6. Click on Start to begin program. The standard thermal shift assay (70 cycles of 1.0C increments) takes ~1:45 to complete.

Exporting/Saving data
1. Bring a USB memory stick to save your data. The software will indicate with a pop-up window if the file completed successfully.
2. The program automatically saves the data into your .sds file. This means that you must repeat the above steps and create a new thermocycler protocol for each run.
3. To export data, first click on the green arrow (Analyze) in the top bar of button. Then go to File, Export, and choose "Delta Rn". This normalizes fluorescence intensity in the first cycle to 0. If you want the raw fluorescence intensities instead, choose "Component". Make sure when saving that you choose the appropriate destination drive!
4. I would recommend also saving the raw data in the .sds form. With these data, you can go back later and reanalyze/export data on any computer with this software (Corey lab has it installed on a PC). To do this, go to File, Save As, and select appropriate destination drive.

Remove plate from thermocycler, turn machine off. Quit software and remove USB drive – the PC won't let you 'safely remove' USB drive until 7500 software is closed.