

IV. Instrument Setup and Thermal Cycling for qPCR and Two-Step qRT-PCR

These instructions describe instrument setup and thermal cycling conditions for DNA or cDNA quantitation using the Plexor® qPCR or Plexor® Two-Step qRT-PCR System. Thermal cycling programs described in this manual are optimized to work with primers designed using the Plexor® Primer Design Software, which can be accessed at: www.promega.com/plexorresources/

Instructions for data export from the SmartCycler® software into the Plexor® Analysis Software are provided in Section VII.

IV.A. Thermal Cycling Program

The thermal cycling program is shown in Table 2. Primers designed using the Plexor® Primer Design Software have an annealing temperature of approximately 60°C.

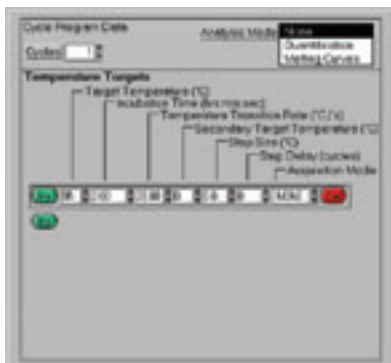
Table 2. qPCR and Two-Step qRT-PCR Thermal Cycling Program.

Step	Temperature	Time	Number of Cycles
Initial denaturation:	95°C	2 minutes	1 cycle
	↓		
Denaturation:	95°C	5 seconds	40 cycles
Annealing and extension:	60°C	35 seconds	
	↓		
Melt temperature curve:	60°C for 5 seconds, ramp 0.4°C/second to 95°C		
Instrument cool	40°C	30 seconds	1 cycle

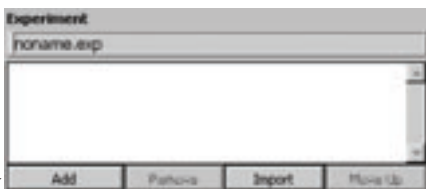
1. Open the LightCycler® software version 3.5.
2. Select "Run" on the opening/front screen.
3. If desired, run the "selftest" when prompted following the instrument instructions.
4. Select "New Experiment" in the Main Window. When prompted, save the file (*.EXP) in the desired location with an appropriate file name.
5. In the dialog box that appears, name the first program of this experiment "Plexor denaturation" or a similar title. Select "OK" .

IV.A. Thermal Cycling Program (continued)

- Ensure that the following “Cycle Program Data” are designated. New segments are added by selecting the lower green “Ins” button.

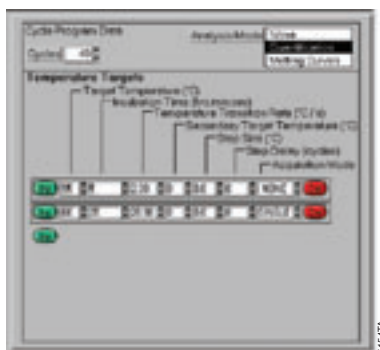


- Select “Add” under the experiment box for a new program.



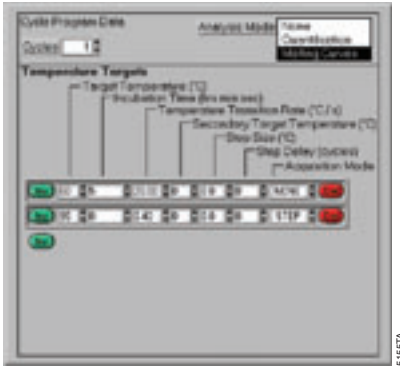
Click “Add” →

- In the dialog box that appears, name the second program of this experiment “Plexor amplification” or a similar title. Select “OK”.
- Change the “Cycle Program Data” to the values indicated below. New segments are added by selecting the lower green “Ins” button.

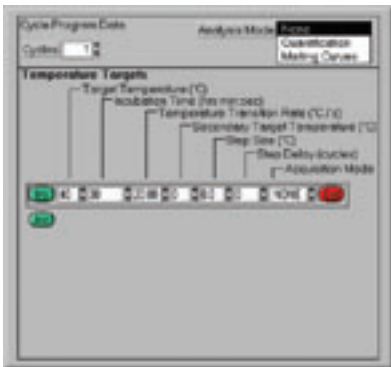


- Select “Add” in the Main Window.
- In the dialog box that appears, name the third program of this experiment “Plexor melt” or a similar title. Select “OK”.

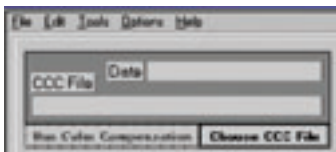
12. Change the “Cycle Program Data” to the values indicated below. New segments are added by selecting the lower green “Ins” button.



13. Select “Add” in the Main Window.
14. In the dialog box that appears, name the fourth program of this experiment “COOL” or a similar title. Select “OK”.
15. Change the “Cycle Program Data” to the values indicated below. New segments are added by selecting the lower green “Ins” button.



16. To apply a color compensation file (see Section III) to a multiplex experiment during setup:
 - a. Select "Choose CCC File" in the Main Window.




- b. Choose the appropriate file from the list that appears.

IV.B. Sample Editing

1. Select “Edit Samples” on the Main Window.
2. In the sample editing window that appears, designate the number of samples in the “Maximum Positions” setting.
3. Name samples if desired. This can be done during the run (by selecting the flashing “Edit Samples” button) or during the Plexor® analysis.
4. Select “Done” when complete.
5. Load the carousel into the LightCycler® instrument.
6. Select the green “Run” button.
7. Enter a name for the experiment data file when prompted, and select “Save”. The “Edit Sample” window will open. Enter the sample names, and select “Done” to start the run.

Note: To start the run before editing the sample names, select “Enter Samples Later”. The sample names can be edited anytime during the run. Once the sample names have been edited, select “Done”.

 Prolonged exposure of the reactions to high temperatures before thermal cycling may adversely affect the final results.

V. Instrument Setup and Thermal Cycling for One-Step qRT-PCR

These instructions describe instrument setup and thermal cycling conditions for cDNA quantitation using the Plexor® One-Step qRT-PCR System. The thermal cycling program includes the initial incubation for the reverse transcription. Thermal cycling programs described in this manual are optimized to work with primers designed using the Plexor® Primer Design Software, which can be accessed at: www.promega.com/plexorresources/

Instructions for data export from the LightCycler® software version 3.5 into the Plexor® Analysis Software are provided in Section VII.