


## V.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.

## VI. Instrument Setup and Thermal Cycling for Genotyping (SNP) Assays

These instructions describe instrument setup and thermal cycling conditions for genotyping assays using the Plexor® qPCR System. The cycling program will include one cycle with an annealing temperature of 50°C, followed by 40 cycles with an annealing temperature of 60°C. The first round of PCR is performed at the lower annealing temperature; additional rounds of PCR are performed at the higher annealing temperature to increase the specificity of amplification. This cycling program is specific for genotyping primers designed using the Plexor® Primer Design Software.

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

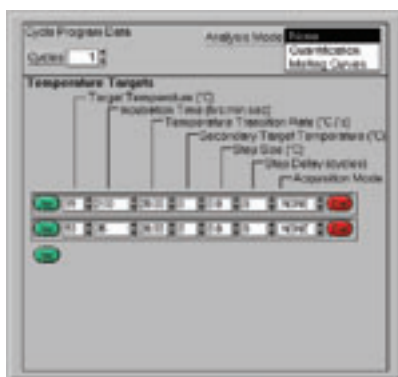
### VI.A. Thermal Cycling Program

The thermal cycling program is shown in Table 4. This program is specific for genotyping primers designed using the Plexor® Primer Design Software. Each of the genotyping primers includes bases that are not complementary to the target sequence at the 5' end. The annealing temperature for the first round of amplification is 50°C to allow the primer to anneal and be extended. Subsequent rounds are performed using an annealing temperature of 60°C, which is the melting temperature ( $T_m$ ) of the primer pair including the noncomplementary bases. The target formed in the first round is amplified during subsequent rounds of amplification, which is performed at the higher annealing temperature to increase specificity.

**Table 4. Thermal Cycling Profile for Genotyping Assays.**

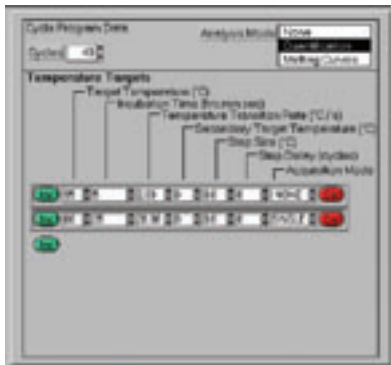
Step	Temperature	Time	Number of Cycles
Initial denaturation:	95°C	2 minutes	1 cycle
Annealing and extension:	50°C	35 seconds	
↓			
Denaturation:	95°C	5 seconds	40 cycles
Annealing and extension:	60°C	35 seconds	
↓			
Melt temperature curve:	60°C for 5 seconds, ramp 0.4°C/second to 95°C.		
↓			
Instrument 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”.
6. Ensure that the following “Cycle Program Data” are designated as shown below. New steps are added by selecting the lower green “Ins” button.



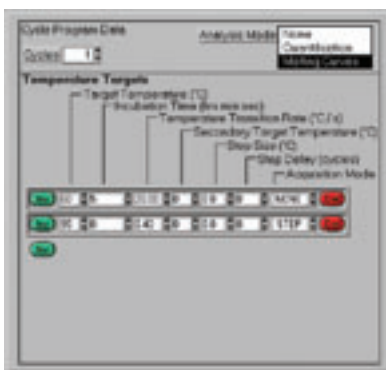
## VI.A. Thermal Cycling Program (continued)

7. Select "Add" in the Main Window.
8. In the dialog box that appears, name the second program of this experiment "Plexor amplification" or a similar title. Select "OK".
9. Change "Cycle Program Data" to the values indicated below. New steps are added by selecting the lower green "Ins" button.



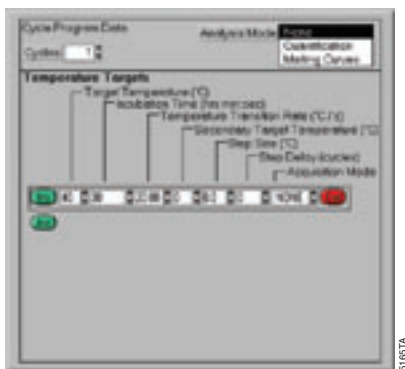
51687A

10. Select "Add" in the Main Window.
11. In the dialog box that appears, name the third program of this experiment "Plexor melt" or a similar title. Select "OK".
12. Change "Cycle Program Data" to the values indicated below. New steps are added by selecting the lower green "Ins" button.



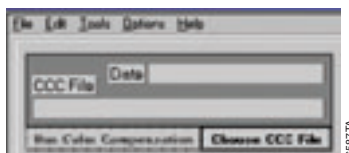
51687A

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 "Cycle Program Data" to the values indicated below. New steps are added by selecting the lower green "Ins" button.



5167A

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.



6887A

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

## VI.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. 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.