

IV.B. Sample Editing (continued)



Sample data

Analysis Type | Reset Samples... | Import SAM... | Auto Copy... | Selected Channels: 530 560 610 640 670 705

Capillary View | Abs Quant | Tm Calling

Sample Count: 16 | LC Carousel ID: | MPLC Batch ID: |
Assay Cat. No.: | Assay Lot No.: | Color Comp ID: |

Pos	Sample Name	Repl. Of	Sample Note
1	1 200 nM 100.000		
2	1 200 nM 10.000		
3	1 200 nM 1.000		
4	1 200 nM MQ		
5	1 400 nM 100.000		
6	1 400 nM 10.000		
7	1 400 nM 1.000		
8	1 400 nM MQ		
9	2 200 nM 100.000		
10	2 200 nM 10.000		
11	2 200 nM 1.000		
12	2 200 nM MQ		
13	2 400 nM 100.000		
14	2 400 nM 10.000		
15	2 400 nM 1.000		
16	2 400 nM MQ		

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Figure 7. List of samples under the “Capillary View” tab. Samples may be named at this point.

3. Name samples if desired. This can be done during analysis with the Plexor™ Analysis Software.
4. Select the appropriate channels for detection (e.g., use 530 for FAM).
Note: When using the LightCycler® 1.5 System with the LightCycler® software version 4.0, select 530 for FAM and 640 for collection of the second dye (i.e., CAL Fluor® Red 610, Texas Red®, HEX, etc.)
5. Load the carousel into the LightCycler® Instrument.
6. Select the green “Run” button.

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. The Plexor™ Primer Design software can be accessed at: www.promega.com/plexorresources/

V.A. Thermal Cycling Program

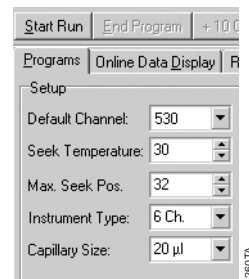
The thermal cycling program is shown in Table 3. Primers designed using the Plexor™ Primer Design Software have an annealing temperature of approximately 60°C.

Table 3. One-Step qRT-PCR Thermal Cycling Program.

Step	Temperature	Time	Number of Cycles
Reverse Transcription ¹ :	45°C	5 minutes	1 cycle
Initial Denaturation and Inactivation of the ImProm-II™ Reverse Transcriptase:	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 to 95°C, ramp 0.4°C/second intervals		
Instrument Cool:	40°C	30 seconds	1 cycle

¹The length of incubation for the reverse transcription reaction can be increased to up to 30 minutes. Longer incubation times can lead to increased sensitivity but also higher background.

1. Open the LightCycler® software version 4.0.
2. Select "Run" in the global toolbar.
3. To apply a color compensation object (Section III.A) to a multiplex experiment during setup:
 - a. Select "Color Compensation" in the "Run" module.
 - b. Choose the appropriate object from the "Select Color Compensation" list that appears.
 - c. Select "OK".
4. In the setup, use the default channel of 530 for FAM. Enter the number of samples to be run next to the "Max. Seek Pos." For multiplex reactions, data are collected for all channels; however, the default channel will be displayed during the experiment and analysis modules.



5. The cycling will consist of four programs. In the “Programs” section, name the first program of this experiment “Reverse Transcription and denaturation/inactivation” or a similar title.
6. Change “Cycle Program Data” to the values indicated below. New steps are added by selecting the lower “+” button.
7. Ensure that the following “Cycle Program Data” are designated:

Add new program

Programs			
Program Name	Cycles	Analysis Mode	
Reverse Transcription and denaturation / inactivation	1	None	
Plexor Amplification	40	Quantification	
Plexor Melt	1	Melting Curves	
COOL	1	None	

Add new step

Reverse Transcription and denaturation / inactivation Temperature Targets						
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode
45	00:05:00	20	0	0	0	None
95	00:02:00	20	0	0	0	None

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8. Select the “+” button next to the program names.
9. In the dialog box that appears, name the second program “Plexor Amplification” or a similar title.
10. Change “Cycle Program Data” to the values indicated here. New steps are added by selecting the lower “+” button.

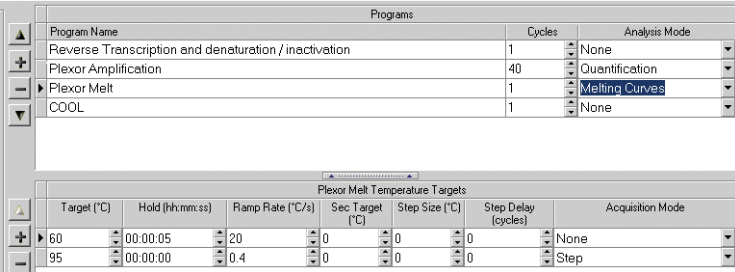
Programs			
Program Name	Cycles	Analysis Mode	
Reverse Transcription and denaturation / inactivation	1	None	
Plexor Amplification	40	Quantification	
Plexor Melt	1	Melting Curves	
COOL	1	None	

Plexor Amplification Temperature Targets						
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode
95	00:00:05	2	0	0	0	None
60	00:00:35	20	0	0	0	Single

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V.A. Thermal Cycling Program (continued)

11. Select the “+” button next to the program names.
12. In the dialog box that appears, name the third program “Plexor Melt” or a similar title.
13. Change “Cycle Program Data” to the values indicated here. New steps are added by selecting the lower “+” button.



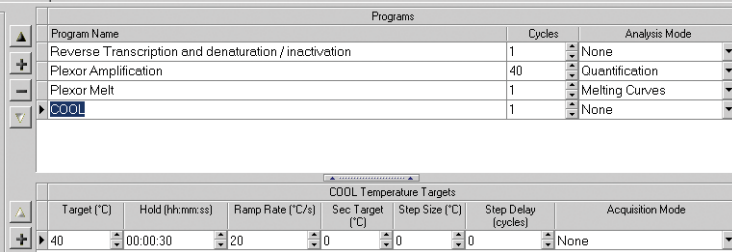
The screenshot shows the software interface with two tables. The top table, titled 'Programs', lists four programs: 'Reverse Transcription and denaturation / inactivation', 'Plexor Amplification', 'Plexor Melt', and 'COOL'. The 'Plexor Melt' program is selected, and its 'Analysis Mode' is set to 'Melting Curves'. The bottom table, titled 'Plexor Melt Temperature Targets', shows two target steps: a 60°C target with a 00:00:05 hold and a 20°C/s ramp rate, and a 95°C target with a 00:00:00 hold and a 0.4°C/s ramp rate. The 'Acquisition Mode' for the 95°C target is set to 'Step'.

Programs							
Program Name	Cycles	Analysis Mode					
Reverse Transcription and denaturation / inactivation	1	None					
Plexor Amplification	40	Quantification					
Plexor Melt	1	Melting Curves					
COOL	1	None					

Plexor Melt Temperature Targets							
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode	
60	00:00:05	20	0	0	0	None	
95	00:00:00	0.4	0	0	0	Step	

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14. Select the “+” button next to the program names.
15. In the dialog box that appears, name the first program of this experiment “cool” or a similar title.
16. Change “Cycle Program Data” to the values indicated below. New steps are added by selecting the lower “+” button.



The screenshot shows the software interface with two tables. The top table, titled 'Programs', lists four programs: 'Reverse Transcription and denaturation / inactivation', 'Plexor Amplification', 'Plexor Melt', and 'COOL'. The 'COOL' program is selected, and its 'Analysis Mode' is set to 'None'. The bottom table, titled 'COOL Temperature Targets', shows one target step: a 40°C target with a 00:00:30 hold and a 20°C/s ramp rate. The 'Acquisition Mode' is set to 'None'.

Programs							
Program Name	Cycles	Analysis Mode					
Reverse Transcription and denaturation / inactivation	1	None					
Plexor Amplification	40	Quantification					
Plexor Melt	1	Melting Curves					
COOL	1	None					

COOL Temperature Targets							
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode	
40	00:00:30	20	0	0	0	None	

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V.B. Sample Editing

Sample information can be entered during or after a run.

1. Select the “Samples” button.
2. The number displayed in the “Sample Count” is the value specified in the “Max. Seek Pos.” field (Section V.A, Step 4). The number of samples cannot be changed once a run has been started.

Sample data			
Analysis Type	Reset Samples...	Import SAM...	Auto Copy...
Capillary View			Selected Channels: 530 560 610 640 670 705
Abs Quant		Tm Calling	
Sample Count	16	LC Carousel ID	MPLC Batch ID
Assay Cat. No.		Assay Lot No.	Color Comp ID
Pos	Sample Name	Repl. Of	Sample Note
1	1 200 nM 100.000		
2	1 200 nM 10.000		
3	1 200 nM 1.000		
4	1 200 nM MQ		
5	1 400 nM 100.000		
6	1 400 nM 10.000		
7	1 400 nM 1.000		
8	1 400 nM MQ		
9	2 200 nM 100.000		
10	2 200 nM 10.000		
11	2 200 nM 1.000		
12	2 200 nM MQ		
13	2 400 nM 100.000		
14	2 400 nM 10.000		
15	2 400 nM 1.000		
16	2 400 nM MQ		

Figure 8. List of samples under the “Capillary View” tab. Samples may be named at this point.

- Name samples if desired. This can be done during analysis with the Plexor™ Analysis Software.
- Select the appropriate channels for detection (e.g., use 530 for FAM).
Note: When using the LightCycler® 1.5 System with the LightCycler® software version 4.0, select 530 for FAM and 640 for collection of the second dye (i.e., CAL Fluor® Red 610, Texas Red®, HEX, etc.)
- Load the carousel into the LightCycler® Instrument.
- Select the green “Run” button.

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. This cycling program is specific for genotyping primers designed using the Plexor™ Primer Design software. Thermal cycling programs described in this manual are optimized to work with primers designed using the Plexor™ Primer Design Software. The Plexor™ Primer Design Software can be accessed at: www.promega.com/plexorresources/