

# Plexor™ Systems Instrument Setup and Data Analysis for the Roche LightCycler® 2.0 and LightCycler® 1.5 Systems using LightCycler® Software Version 4.0

All technical literature is available on the Internet: at [www.promega.com/tbs/](http://www.promega.com/tbs/)  
 Please visit the web site to verify that you are using the most current version of this Technical Manual.

<b>I. Description</b> .....	2
<b>II. Capillary Preparation and Amplification</b> .....	2
<b>III. Generating the Color Compensation Object</b> .....	3
A. Color Compensation Thermal Cycling Program .....	3
B. Color Compensation Sample Editing .....	6
C. Dye Calibrator Setup .....	7
<b>IV. Instrument Setup and Thermal Cycling for qPCR and Two-Step qRT-PCR</b> .....	8
A. Thermal Cycling Program .....	8
B. Sample Editing .....	11
<b>V. Instrument Setup and Thermal Cycling for One-Step qRT-PCR</b> .....	12
A. Thermal Cycling Program .....	13
B. Sample Editing .....	16
<b>VI. Instrument Setup and Thermal Cycling for Genotyping (SNP) Assays</b> .....	17
A. Thermal Cycling Program .....	18
B. Sample Editing .....	21
<b>VII. Data Export from the LightCycler® Data Analysis Software</b> .....	22
<b>VIII. Data Import into the Plexor™ Analysis Software</b> .....	26
<b>IX. Data Analysis with the Plexor™ Analysis Software</b> .....	30
A. Sample Definition.....	30
B. Adjusting the Expected Target Melt Temperature .....	32
C. Adjusting the Y Axes of the Amplification and Thermal Melt Curves (Optional) .....	34
D. Adjusting the Baseline Region and Melt Threshold Line (Optional) .....	34
E. Generating a Standard Curve (Optional) .....	35
F. Reports .....	38
G. Saving and Printing the Analysis File.....	40
<b>X. Troubleshooting</b> .....	40
<b>XI. Appendix</b> .....	47

A.	Plexor™ Analysis Software Operating System Compatibility .....	47
B.	Plexor™ Analysis Software Installation .....	47
C.	Advanced Options .....	47
D.	Manual Baseline Adjustments.....	50
E.	Icon Definitions .....	51
F.	Amplification Efficiency Calculations .....	53
G.	Reference .....	53

## I. Description

The Plexor™ qPCR and qRT-PCR Systems<sup>(a,b,c)</sup> are compatible with a variety of real-time PCR instruments. Data from these instruments can be analyzed with one dedicated software program, the Plexor™ Analysis Software. This manual includes instructions and thermal cycling conditions specific for use of the Plexor™ qPCR System, Plexor™ One-Step qRT-PCR System and Plexor™ Two-Step qRT-PCR System with the Roche LightCycler® 2.0 and 1.5 Systems using LightCycler® software version 4.0. Instructions are included for instrument setup, data transfer from the instrument to the Plexor™ Analysis Software and data analysis.

## II. Capillary Preparation and Amplification

Detailed instructions describing assay setup are provided in the *Plexor™ qPCR System Technical Manual #TM262*, *Plexor™ One-Step qRT-PCR System Technical Manual #TM263* or *Plexor™ Two-Step qRT-PCR System Technical Manual #TM264*.

When using the Plexor™ qPCR System for the first time, we recommend programming the thermal cycling conditions and checking that the instrument is compatible with the dyes used and is configured and calibrated (color compensation file generated) for those dyes before assembling the reactions, so the reactions are not kept on ice for prolonged periods of time. Once you are familiar with the programming process, the instrument can be programmed after reaction assembly.

### Materials to Be Supplied By the User

- carousel centrifuge or adapter and centrifuge
  - capillary seals (plastic stoppers)
1. Program the Roche LightCycler® 2.0 or 1.5 System with software version 4.0. The proper thermal cycling conditions and instructions for programming the instrument using version 4.0 are provided in Section IV (qPCR and two-step qRT-PCR assays), Section V (one-step qRT-PCR assays) and Section VI (genotyping assays). Section III describes instrument setup for color compensation, a necessary control for multiplex assays on the Roche LightCycler® 2.0 System.

**Note:** For the LightCycler® 1.5 with earlier software versions, see Technical Manual #TM269

2. After the reactions have been assembled in capillaries, seal capillaries and centrifuge briefly following the instrumentation instructions to collect contents at the bottom of each capillary.

**Note:** Keep the reactions on ice during reaction setup until loading the LightCycler® carousel.

### III. Generating the Color Compensation Object

For multiplex assays, a color compensation object (cc object) must be created and applied to multicolor data to allow proper interpretation. To generate the cc object, labeled Plexor™ primers are used as dye calibrators in an initial color compensation cycling experiment. Any subsequent runs performed with the same dyes and similar cycling conditions can be analyzed using this color compensation object.

#### Notes:

1. The LightCycler® instrument should be programmed before preparing the Plexor™ primers that will be used as the dye calibrators (Section III.D).
2. The color compensation object can be generated after the experimental run and applied to the multiplex experiment before final analysis.
3. A list of LightCycler®-compatible dyes is available at:  
[www.promega.com/plexorresources/](http://www.promega.com/plexorresources/)

#### III.A. Color Compensation Thermal Cycling Program

1. Open LightCycler® software (version 4.0).
2. Select the “Run” module from the global toolbar.
3. Select the “Programs” tab.
4. Under “Setup,”
  - a. Set “Default Channel” to “530.”
  - b. Set the “Seek Temperature” to “30.”
  - c. Set “Max. Seek Pos.” equal to the number of capillaries to be used. This will be equal to the number of dyes in the calibration plus one for the calibrator blank.
  - d. Set “Instrument Type” to “6 Ch.” (switch to “3 Ch.” for LightCycler® 1.5 using software version 4.0).
  - e. Set the “Capillary Size” to “20µl”. Adjust the volume accordingly if other capillary sizes are used.

