

# Plexor<sup>®</sup> Systems Instrument Setup and Data Analysis for the Roche LightCycler<sup>®</sup> 1.0 and LightCycler<sup>®</sup> 1.5 Systems using LightCycler<sup>®</sup> Software Version 3.5



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

|       |   |    |
|-------|---|----|
| I.    | Description.....  | 2  |
| II.   | Capillary Preparation and Amplification.....  | 2  |
| III.  | Generating the Color Compensation File.....   | 3  |
|       | A. Color Compensation Thermal Cycling Program .....                                   | 3  |
|       | B. Color Compensation Sample Editing .....  | 6  |
|       | C. Dye Calibrator Setup .....   | 7  |
|       | D. Converting Experiment to Color Compensation File (CCC File).....                   | 8  |
| IV.   | Instrument Setup and Thermal Cycling for qPCR and Two-Step qRT-PCR .....              | 9  |
|       | A. Thermal Cycling Program .....  | 9  |
|       | B. Sample Editing .....   | 12 |
| V.    | Instrument Setup and Thermal Cycling for One-Step qRT-PCR.....                        | 12 |
|       | A. Thermal Cycling Program .....  | 13 |
|       | B. Sample Editing .....   | 16 |
| VI.   | Instrument Setup and Thermal Cycling for Genotyping (SNP) Assays.....                 | 16 |
|       | A. Thermal Cycling Program .....  | 16 |
|       | B. Sample Editing .....   | 19 |
| VII.  | Data Export from the LightCycler <sup>®</sup> Data Analysis Software.....             | 20 |
| VIII. | Data Import into the Plexor <sup>®</sup> Analysis Software .....                      | 22 |
| IX.   | Data Analysis with the Plexor <sup>®</sup> Analysis Software.....                     | 25 |
|       | A. Sample Definition.....   | 25 |
|       | B. Adjusting the Expected Target Melt Temperature .....                               | 28 |
|       | C. Adjusting the Y Axes of the Amplification and Thermal Melt Curves (Optional) ..... | 29 |
|       | D. Adjusting the Baseline Region and Amplification Threshold Line (Optional) .....    | 30 |
|       | E. Generating a Standard Curve (Optional) .....                                       | 31 |
|       | F. Reports .....  | 33 |
|       | G. Saving and Printing the Analysis File.....   | 35 |
| X.    | Troubleshooting.....  | 35 |



|   |    |
|---|----|
| XI. Appendix .....  | 42 |
| A. Plexor® Analysis Software Operating System Compatibility ..... | 42 |
| B. Plexor® Analysis Software Installation .....                   | 42 |
| C. Advanced Options .....   | 42 |
| D. Manual Baseline Adjustments.....                               | 45 |
| E. Icon Definitions .....   | 46 |
| F. Amplification Efficiency Calculations .....                    | 48 |
| G. Reference .....  | 48 |

## I. Description

The Plexor® qPCR and qRT-PCR Systems<sup>(a-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® 1.0 and 1.5 Systems with version 3.5 software. 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*. Additional information is available at: [www.promega.com/plexorresources/](http://www.promega.com/plexorresources/)

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 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
  - plastic stoppers to seal the capillaries
1. Program the Roche LightCycler® System. The proper thermal cycling conditions and instructions for programming the instrument 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 LightCycler® 1.0 system.
  2. After the amplification 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 and programming of the thermal cycling conditions.