LightCycler® 480 Instrument Quick Guide

For life science research use only.
Not for use in diagnostic procedures.

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Revision History

Publication Reference Number	Date	Revision Purpose
5125-00-1113	November 2013	New Document Creation

General Required Materials

Standard Laboratory Equipment

Product Name
Microwell Plate Centrifuge
Nuclease-free, aerosol-resistant pipette tips
Microcentrifuge and 1.5 mL microcentrifuge tubes
Micropipettes (P10 or P20, P200, P1000 or equivalent)

Disposables

Product Name	Catalog Number	Description
LightCycler® 480 Multi-well Plate 96-well - White	04 729 692 001	50 plates and sealing foils
LightCycler® 480 Multi-well Plate 384-well - White	04 729 749 001	50 plates and sealing foils
LightCycler® 480 Multi-well Plate 96-well - Clear	05 102 413 001	50 plates and sealing foils
LightCycler® 480 Multi-well Plate 384-well - Clear	05 102 430 001	50 plates and sealing foils
LightCycler® 480 Sealing Foil	04 729 757 001	50 sealing foils

Reagents

Reaction Type	Product Name	Catalog Number	Description
Reverse Transcription with RNAse H activity	Transcriptor 1st Strand cDNA Synthesis Kit	04 379 012 001	For 50 (20 µL) reactions
SYBR Green Master	LightCycler® 480 SYBR Green I Master	04 707 516 001	5 mL (5 x 20 µL) 2x concentrated Master Mix for 250 (20 µL) reactions
High Resolution Melting	LightCycler® 480 High Resolution Melting Master	04 909 631 001	5 mL (5 x 1 mL) 2x concentrated 25 mL MgCl2 stock solution for 500 (20 µL) reactions
Hybridization Probes	LightCycler® 480 Genotyping Master	04 707 524 001	1.5 mL 5x concentrated Master Mix for 250 (20 µL) reactions
Hydrolysis Probes *TaqMan® Probes or Universal Probe Library (UPL)*	LightCycler® 480 Probes Master	04 707 494 001	5 mL (5 x 1 mL) 2x concentrated Master Mix for 250 (20 µL) reactions
	LightCycler® 480 Control Kit	04 710 924 001	Kit for quantitative Real-Time PCR and genotyping reactions

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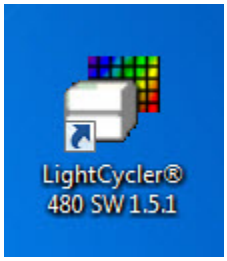
Getting Started

1. Log on to the LightCycler® computer.

   Username: Operator

   Password: LC480

2. Start the LightCycler® 480 software by double-clicking the <**LightCycler480**> icon.



3. Enter the username and assigned password to log in to the LightCycler® 480 software.

   First Time Login (new database)

   Username: admin

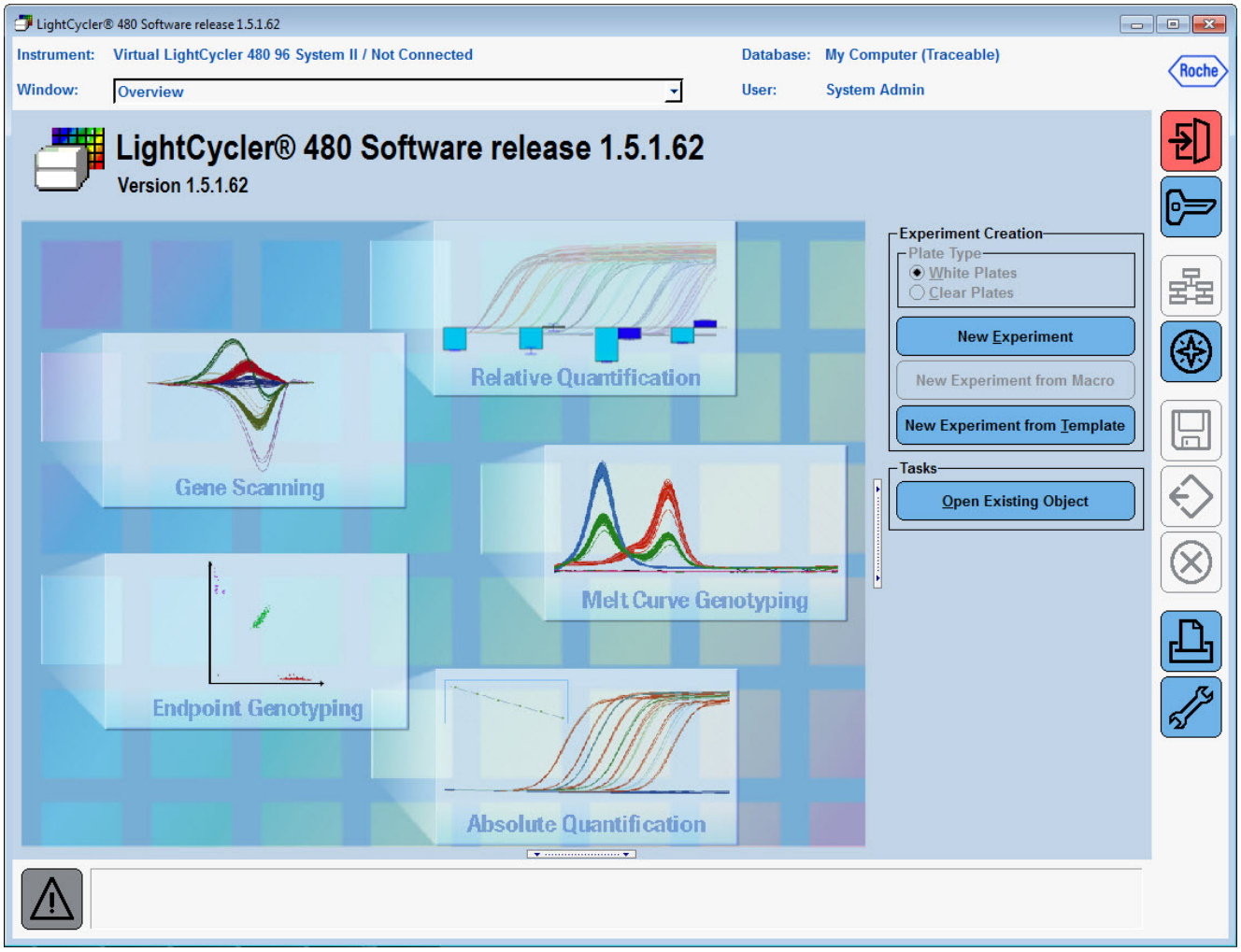
   Password: LightCycler480

   Normal Login

   Username: admin

   Password: Roche480

4. The overview screen displays. This screen allows entry of a new experiment with or without use of a template for the conditions, or run a previously programed macro.



Software Navigation Overview

Exit the software

Logout of the current database

Switch to Overview menu

Display Navigator window

Save the current experiment

Export the current experiment to an ".ixo" file

Close the current experiment

Print the current screen

Display Tools window

Navigator window: displays the objects stored in the database as folders and files, allowing the user to work with objects in the database as well as import and export

Exporting as .ixo: creates a copy which can be imported to another computer or database

Tools window: allows user to change passwords, edit system settings, view database status and Error Log, define filter combination settings

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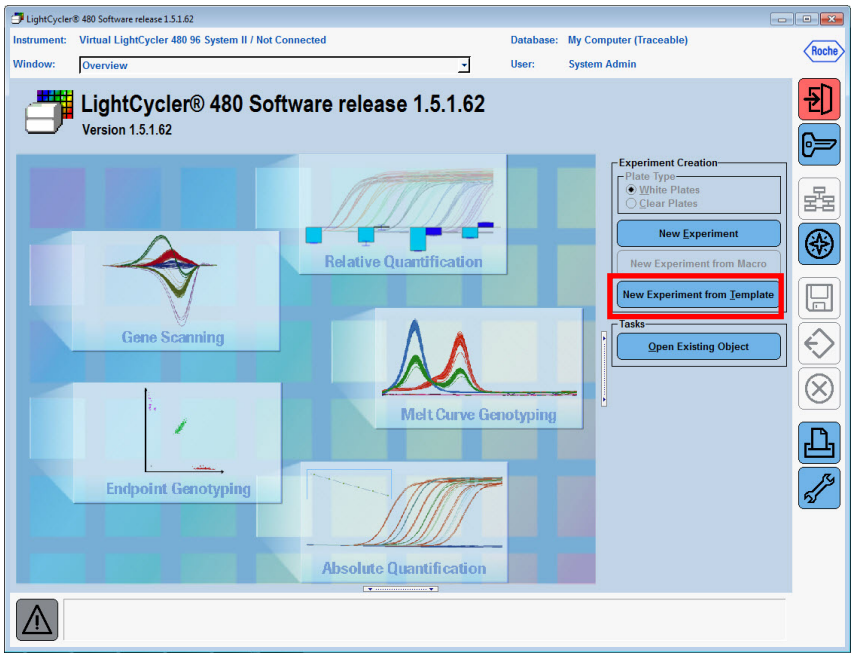
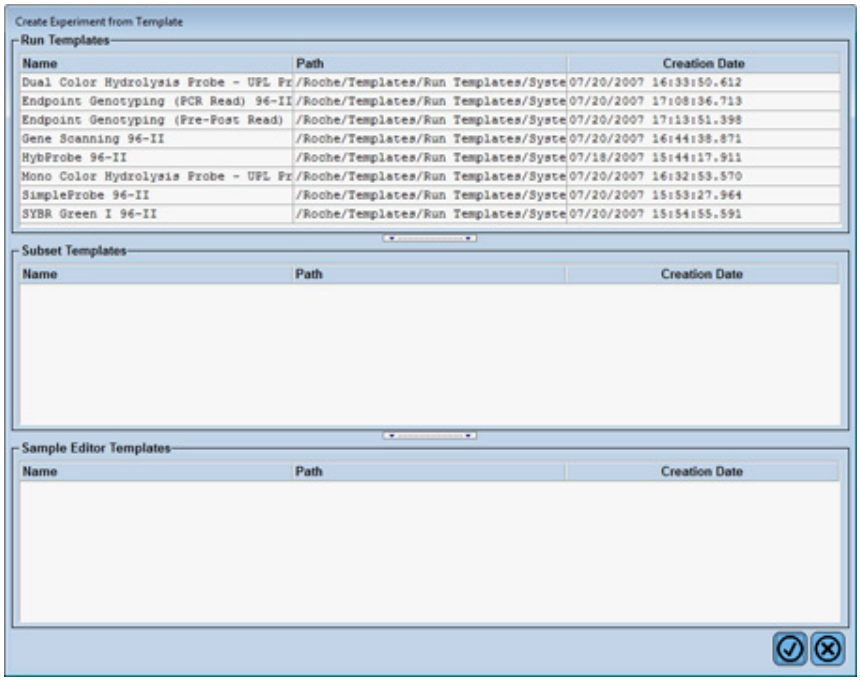
Create a New Experiment from Template

Templates provide a convenient way to speed up the process of creating an experiment. You can use Roche general templates for the different types of Real-time PCR reactions, as well as create your own templates based on the specific conditions of your experiments.

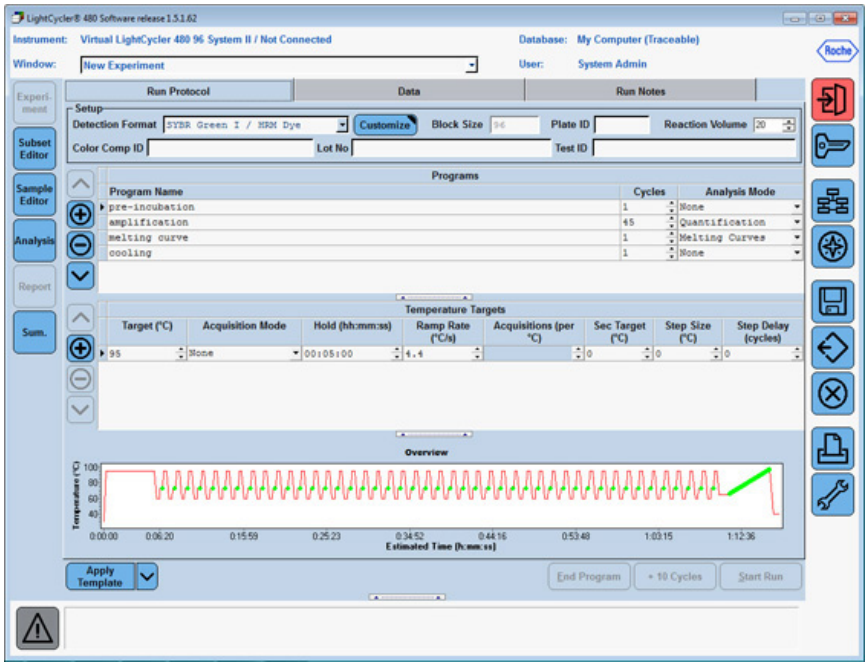
1. Click **New Experiment from Template** in the *Overview* window.



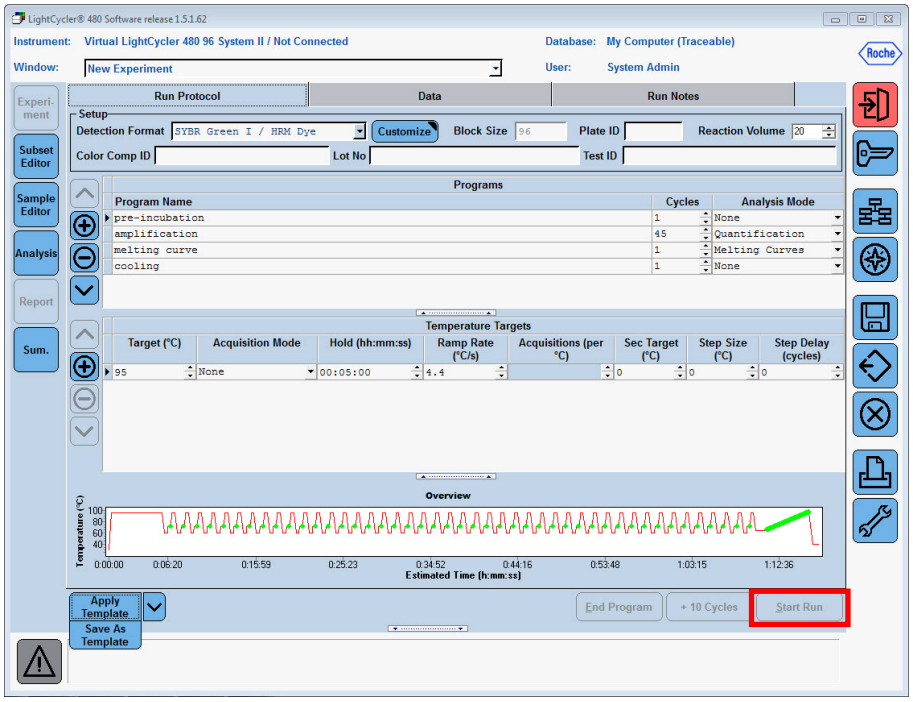
2. The *New Experiment from Template* window displays the templates that match the connected instrument.



3. Select a run template.
    a. Dual Color Hydrolysis Probes/UPL Probe
        - duplex reactions using Hydrolysis Probes (UPL/TaqMan) labeled with FAM and VIC, HEX or Yellow 555
    b. End-point Genotyping (PCR read)
        - genotyping with FAM and VIC or HEX-labeled Hydrolysis or TaqMan probes, PCR and data collection (Allelic discrimination)
    c. End-point Genotyping (Pre-Post read)
        - genotyping with FAM and VIC or HEX-labeled probes, data collection only, PCR performed in another instrument (Allelic Discrimination)
    d. Gene Scanning
        - basic protocol for High Resolution Melting experiments, including touch-down protocol
    e. HybProbe
        - reactions using Hybridization Probes, labeled with Fluorescein and LightCycler Red 640
    f. Mono Color Hydrolysis Probe/UPL
        - reactions with Hydrolysis Probes (UPL/TaqMan) labeled with FAM
    g. Simple Probes
        - melting curve genotyping reactions using a single Fluorescein-labeled probe (Simple Probe)
    h. SYBR Green
        - reactions using SYBR Green and simliar double-strand-intercalating dyes, includes melting curve
4. Click  . The selected run-template is applied.
5. Modify run conditions (reaction volume, number of cycles, target temperature, hold, ramp rate, etc.) as needed.



6. The "*Start Run*" icon becomes active once the multi-well plate is loaded in the instrument.



7. Click **Start Run**.

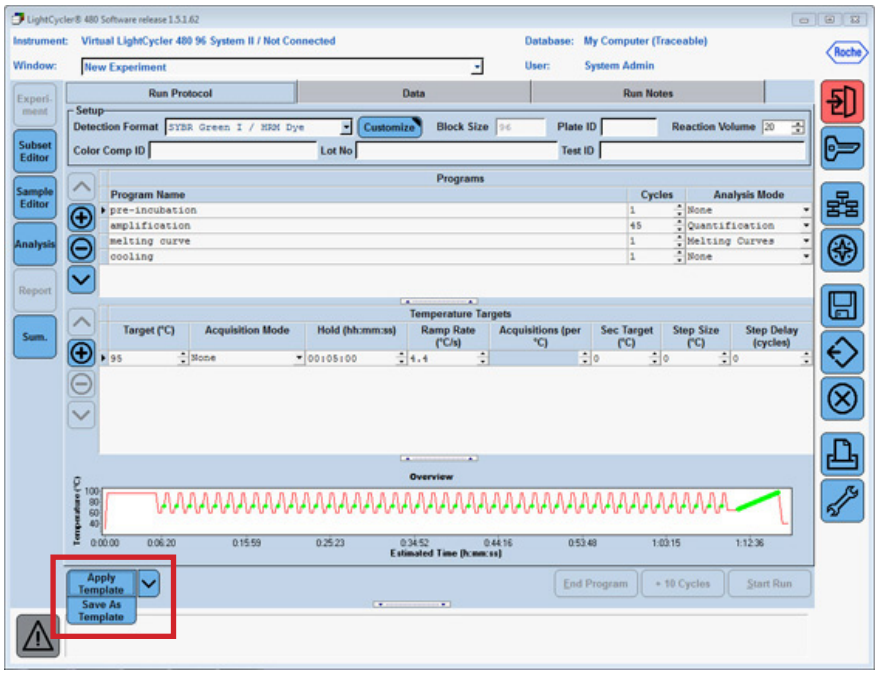
8. Enter the experiment name in the dialog box.

Save Run Conditions as a Template

If you modify the run conditions, you can save it as a new template for future use.

1. Open or create the run to be used as a template.

2. Click the drop-down next to "*Apply Template*" and select **Save as Template**.



3. Name the run template and select a location to save the template.

   Note: *The default storage location for templates is the user's Template folder.*

4. Click  . The template is saved and will appear in the *New Experiment from Template* window in the future.

Typical SYBR Green Reaction Protocol

Reaction Setup

Reagent	*Final Concentration*	*Volume (µL) / one reaction*
Water		1.0
Primer - Forward (5 µM Stock)	0.5 µM	2.0
Primer - Reverse (5 µM Stock)	0.5 µM	2.0
LightCycler® 480 SYBR Green I Master	1x	10.0
	Total	15.0
Add 5 µL template DNA for a total volume of 20 µL		

Run Program

Program Name	**Cycles**	**Analysis Mode**
Pre-incubation	1	None

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*
95	None	00:05:00

Program Name	**Cycles**	**Analysis Mode**
Amplification	45	Quantification

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*
95	None	00:00:10
60	None	00:00:10
72	Single	00:00:10

Program Name	**Cycles**	**Analysis Mode**
Melting Curve	1	Melting Curves

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*	*Acquisitions (per °C)*
95	None	00:00:05	
65	None	00:01:00	
97	Continuous		5

Program Name	**Cycles**	**Analysis Mode**
Cooling	1	None

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*
40	None	00:00:30

Typical Mono Color Hydrolysis Probe *(TaqMan®/UPL)* Reaction Protocol

Reaction Setup

Reagent	*Final Concentration*	*Volume (µL) / one reaction*
Water		0.6
Primer - Forward (5 µM Stock)	0.5 µM	2.0
Primer - Reverse (5 µM Stock)	0.5 µM	2.0
Probe (10 µM Stock)	0.2 µM	0.4
LightCycler® 480 Probe Master	1x	10.0
	Total	15.0
Add 5 µL template DNA for a total volume of 20 µL		

Run Program

Program Name	**Cycles**	**Analysis Mode**
Pre-incubation	1	None

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*
95	None	00:10:00

Program Name	**Cycles**	**Analysis Mode**
Amplification	45	Quantification

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*
95	None	00:00:10
60	Single	00:00:30
72	None	00:00:01

Program Name	**Cycles**	**Analysis Mode**
Cooling	1	None

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*
40	None	00:00:30

Typical Hybridization Probe (HybProbe) Reaction Protocol

Reaction Setup

Reagent	*Final Concentration*	*Volume (µL) / one reaction*
Water		6.2
Primer - Forward (5 µM Stock)	0.5 µM	2.0
Primer - Reverse (5 µM Stock)	0.5 µM	2.0
Fluorescein Probe (10 µM Stock)	0.2 µM	0.4
Red Fluor Probe (10 µM Stock)	0.2 µM	0.4
LightCycler® 480 Genotyping Master	1x	4.0
	Total	15.0
Add 5 µL template DNA for a total volume of 20 µL		

Run Program

Program Name	**Cycles**	**Analysis Mode**
Pre-incubation	1	None

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*
95	None	00:10:00

Program Name	**Cycles**	**Analysis Mode**
Amplification	45	Quantification

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*
95	None	00:00:10
60	Single	00:00:10
72	None	00:00:10

Program Name	**Cycles**	**Analysis Mode**
Melting Curve	1	Melting Curves

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*	*Acquisitions (per °C)*
95	None	00:01:00	
40	None	00:02:00	
95	Continuous		5

Program Name	**Cycles**	**Analysis Mode**
Cooling	1	None

Target (°C)	*Acquisition Mode*	*Hold (hh:mm:ss)*
40	None	00:00:30

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