

1) Start the Instrument and Software	2
1.1 Start the Instrument	2
1.2 Load Your Plate	3
1.3 Start the Software	4
2) Setup the Software to Run a New Experiment	5
2.1 Setup a New Experiment	5
2.2 Apply a Template	6
2.3 Modify the Template to Fit Your Experiment	6
2.4 Save your Program as Template	8
3) Enter Sample Names	9
3.1 Tips on Entering Sample Names	9
3.2 Save As a Template	9
4) Start the Run and Online Data Display	10
4.1 Start The Run	10
4.2 During The Run	10
5) Appendix A: General Information	11
5.1 Ordering Information for SYBR Green Assay	11
5.2 Reaction Set-Up	11
5.3 Reaction Volumes	11
5.4 Complete Roche qPCR Product & Price List	12

## 1) Start the Instrument and Software

### 1.1 START THE INSTRUMENT

1. Turn on the main switch located on the left side of the power box at the back of the LightCycler 480 instrument.



2. The two status lights on the front of the instrument indicate the status of the machine. When the color of the left LED changes to green and the right LED shows a steady orange (takes about 3½ minutes), the instrument is ready. You may load your plate at any time now. **Note- to avoid sample evaporation, make sure your plate is sealed properly with optical adhesive film.**

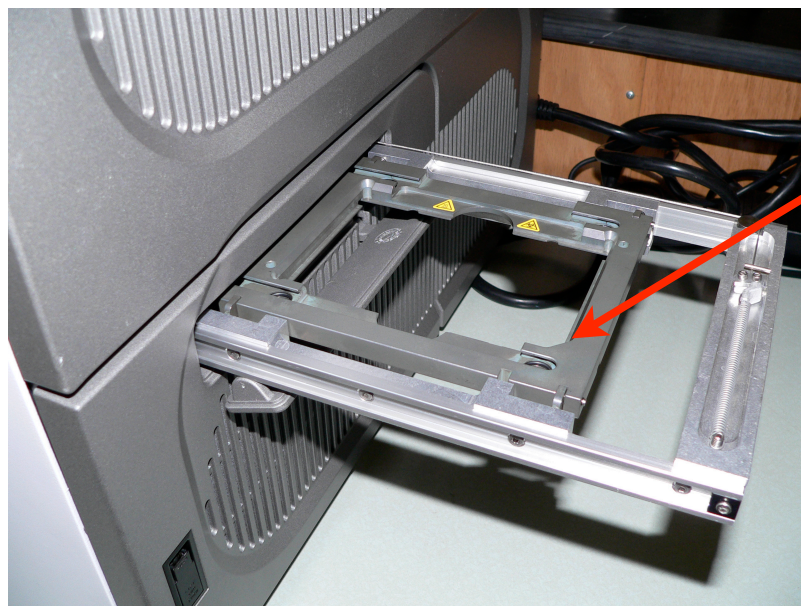
Color of Left LED	Color of Right LED	Status of Instrument
Orange *flashing*	Orange *flashing*	Instrument is initializing.
Green	Orange	Instrument is on and ready. No plate is loaded.
Green	Orange *flashing*	Plate is loading
Green	Green	Instrument is on and ready. Plate is loaded.
Green *flashing*	Green *flashing*	Instrument is running.

## 1.2 LOAD YOUR PLATE

1. To load your plate, press the button on the front of the instrument located next to the status LEDs.



2. The multiwell plate loader will extend out of the right side of the instrument. Place your plate into the frame of the loader with the flat edge of the plate pointing towards the instrument. The short plate edge with the beveled corner points away from the instrument.



Beveled corner  
goes here

3. Press the plate-loading button again to retract the plate loader back into the instrument. The instrument status LED's should change to **green, green**.

### 1.3 START THE SOFTWARE

1. Turn on the computer workstation (if it is not already on).

2. Login to Windows.

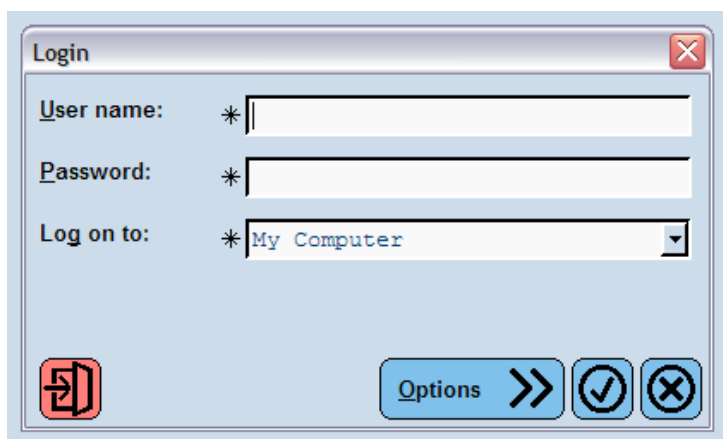
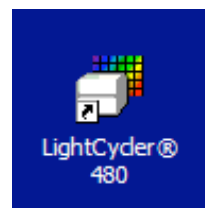
a. User name: operator

b. Password: LC480

3. Start the LightCycler Software

a. Double-click the LightCycler 480 icon on the desktop.

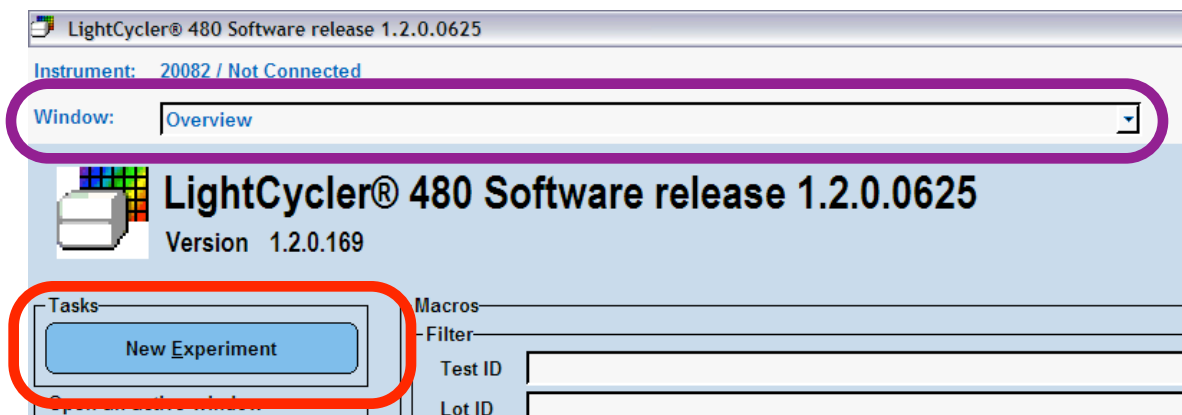
b. Login to the software. If you do not know your User Name and Password, please contact the facility manager (Amy Beaven, [abeaven@umd.edu](mailto:abeaven@umd.edu), x5-7238).



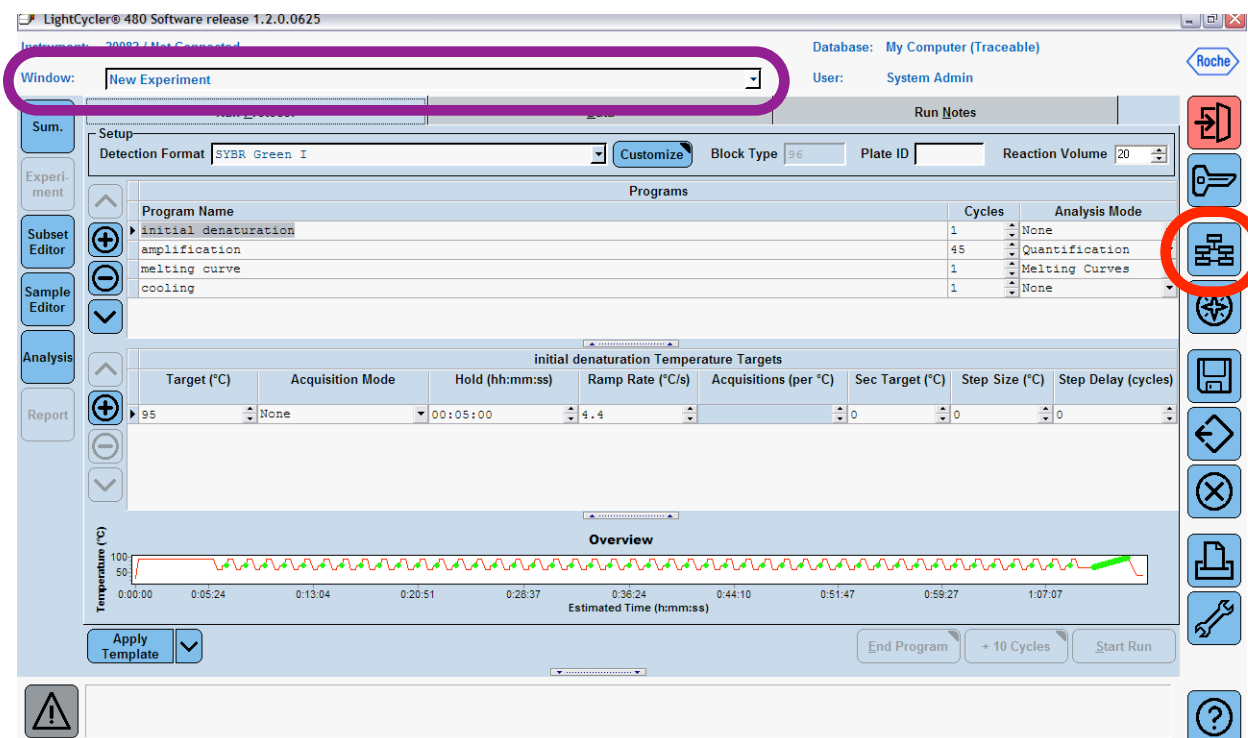
## 2) Setup the Software to Run a New Experiment

### 2.1 SETUP A NEW EXPERIMENT


1. When you start up the software, the **Overview Window** will appear. Click on **New Experiment**.

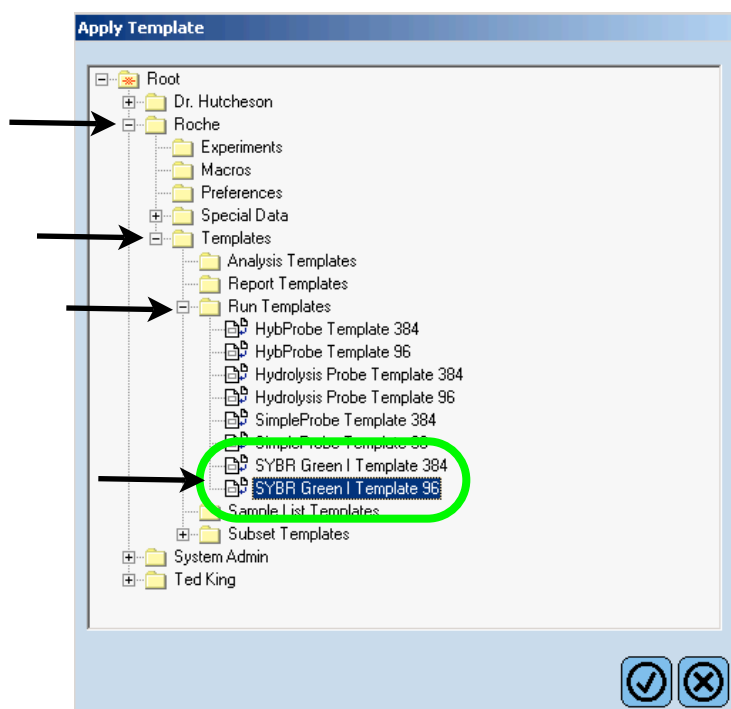


2. The software will open the **New Experiment Window**. If you need to return to the Overview Window at any time, select the **Overview** button from the menu on the right-hand side of the screen.



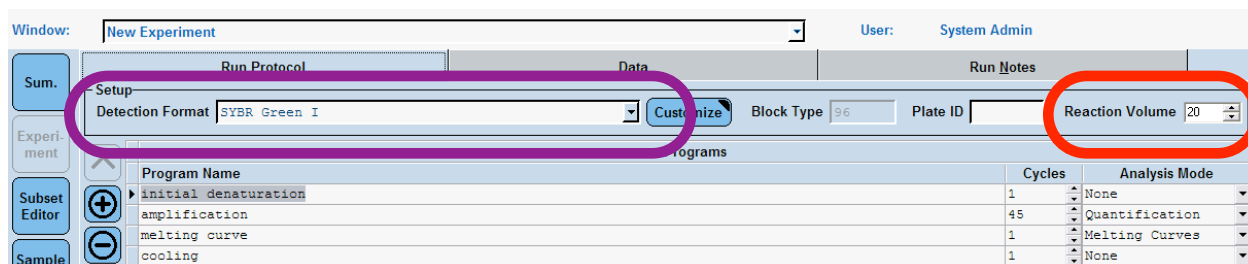
## 2.2 Apply a Template

1. The easiest way to set up a SYBR Green experiment for the first time is to apply a pre-configured template and then modify it as needed.
2. To apply a template, click on **Apply Template** at the bottom left-hand side of the Experiment window. 
3. Navigate to the Templates folder and select **SYBR Green 1 Template 96** for a 96-well plate set-up or **SYBR Green 1 Template 384** for a 384-well set-up.





## 2.3 MODIFY THE TEMPLATE TO FIT YOUR EXPERIMENT

1. In the New Experiment window, the **Detection Format** should now read SYBR Green I.
2. Enter your **Reaction Volume**. The possible range of reactions volumes are 10-100µl for a 96-well plate and 5-20µl for a 384-well plate.





- Note- the following protocol is optimized for the LightCycler® 480 SYBR Green 1 Master Mix available from Roche. Always check the instructions included in your own SYBR Green Master Mix for recommendations on experimental protocol.
- A typical SYBR Green experiment will have four separate **Programs** - initial denaturation, amplification, melt curve (also called a dissociation curve) and cooling. Each program is repeated for a certain number of **Cycles**. The **Analysis Mode** indicates the expected analysis type for the program. To add a new program, click the . To delete a program, click the .

Program Name	Cycle	Analysis Mode
initial denaturation	1	None
amplification	45	Quantification
melting curve	1	Melting Curves
cooling	1	None

- Each program consists of one or more temperature targets. A temperature target specifies the **Target temperature** that is to be reached, the *duration* (**'Hold'**) for which the temperature is applied, and the *speed* (**'Ramp Rate'**) with which the temperature is reached. The **Acquisition Mode** indicates the frequency with which data is acquired. Click on the **Program Name** to show the Target temperature, Acquisition Mode, Hold and Ramp Rate for each program.

Program Name	Cycles	Analysis Mode
initial denaturation	1	None
amplification	45	Quantification
melting curve	1	Melting Curves
cooling	1	None

initial denaturation Temperature Targets							
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:05:00	4.4	0	0	0	0

- During the **Initial Denaturation** program, the temperature will increase at a rate of 4.4°C/s until it reaches 95°C, where it is held for 5 minutes. There is only one cycle in this step and no data is collected (Acquisition Mode = None). This step activates the Taq DNA polymerase and denatures the DNA.

Program Name	Cycles	Analysis Mode
initial denaturation	1	None
amplification	45	Quantification
melting curve	1	Melting Curves
cooling	1	None

initial denaturation Temperature Targets							
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:05:00	4.4	0	0	0	0

7. During the **Amplification** program, the temperature is first increased to 95°C at a rate of 4.4°C/s, where it is held for 10 seconds in order to allow the DNA to denature. *Note* - The target temperature of the next step is primer-dependent. In this example, the temperature is ramped down to 60°C at a rate of 2.2°C/s and held for 10 seconds. During this step, the primers bind to the DNA. For initial experiments, it is a good idea to set the target temperature 5°C below the primer  $T_m$ . The temperature is then increased to 72°C at a rate of 4.4°C and held for 10 seconds. During this time, the target DNA is synthesized. Note that during this step, the acquisition mode is set to single. This means that fluorescence data is acquired once at the end of the temperature segment in each cycle. This sequence is repeated 45 times in order to amplify the target DNA.

Program Name		Cycles	Analysis Mode
initial denaturation		1	None
► amplification		45	Quantification
melting curve		1	Melting Curves
cooling		1	None

amplification Temperature Targets							
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
► 95	None	00:00:10	4.4		0	0	0
60	None	00:00:10	2.2		0	0	0
72	Single	00:00:10	4.4		0	0	0

8. Following amplification, a **Melt curve** is generated in order to assess the specificity of the amplified PCR product. The DNA is first denatured by holding the temperature at 95°C for 5 seconds and then re-annealed by lowering the temperature to 65°C for 1 minute. The fluorescence signal is then monitored continuously as the temperature is increased in small increments. As the DNA denatures ('melts'), the fluorescence decreases.

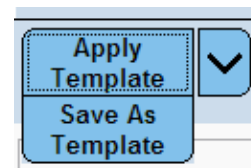
Programs			
Program Name	Cycles	Analysis Mode	
initial denaturation	1	None	
amplification	45	Quantification	
melting curve	1	Melting Curves	
cooling	1	None	

melting curve Temperature Targets							
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:05	4.4				
65	None	00:01:00	2.2				
97	Continuous			5			

9. The final cooling program is not necessary to complete the experiment, but is included as a safety precaution.

## 2.4 Save your Program as Template

1. To save your program as a template, click on **Save As Template** at the bottom left-hand side of the Experiment window.

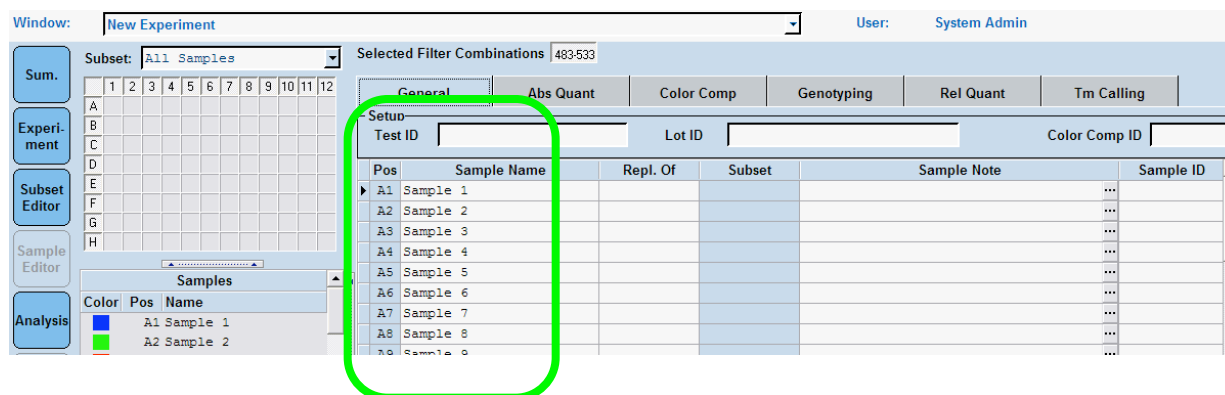
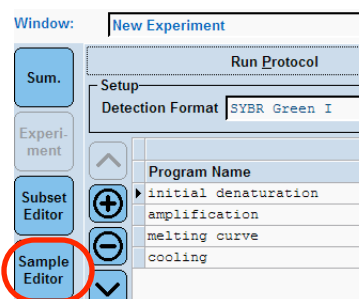




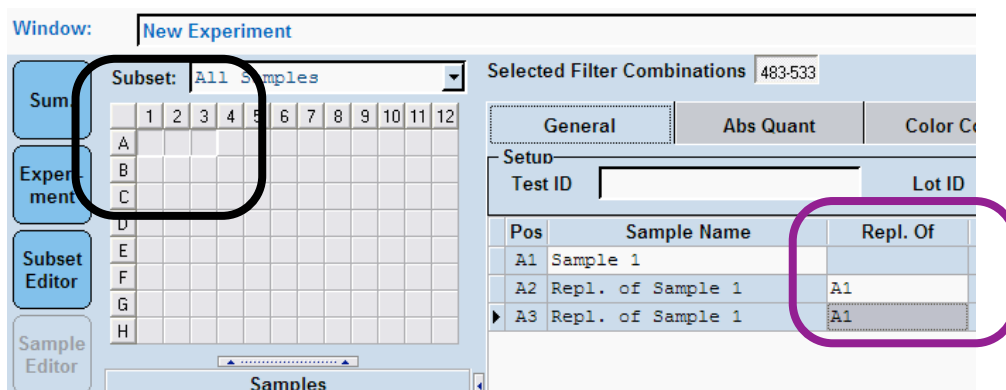
### 3) Enter Sample Names

#### 3.1 TIPS ON ENTERING SAMPLE NAMES

1. Note- Data entry can be done before or after the run.
2. Select **Sample Editor** from the left-hand side of the New Experiment window.
3. Under the General tab, enter in the names of your samples in the **Sample Name** column manually or by copying and pasting from Excel.

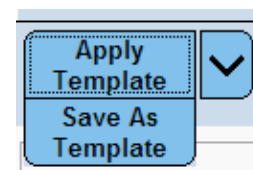


4. To enter sample names more easily, select samples by **highlighting** specific wells. Indicate replicates in the **Repl. Of** column.



#### 3.2 SAVE AS A TEMPLATE


1. To save your plate set-up as a template for a future run, click on **Save As Template** at the bottom left of the screen.

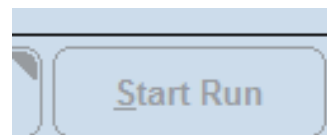


## 4) Start the Run and Online Data Display

---

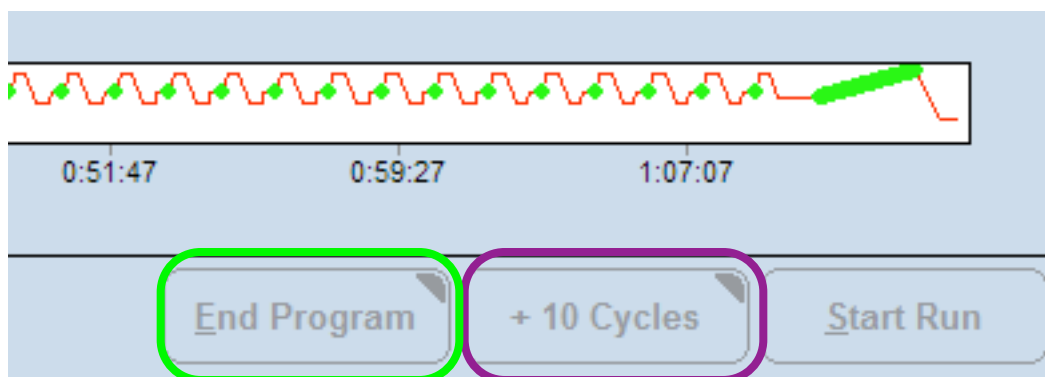
### 4.1 START THE RUN

1. Go back to the **Experiment window** and click on **Start Run** at the bottom right-hand corner of the screen. Note- you cannot start a run unless a plate has been loaded. Save your experiment when prompted- enter your experiment name and select a folder to save it in.
2. When the run starts, the display automatically switches to the online data display mode and 'Start Run' changes to 'Abort Run'.
3. *Note* - Click on the  tab to access different display options.

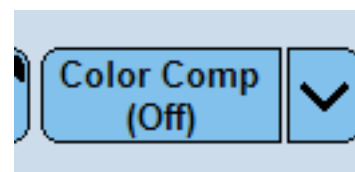


### 4.2 DURING THE RUN

1. Add Cycles: To add cycles to a run, click on **+10 Cycles** while in the amplification or cycling program.



2. End Program: To end the program without losing collected data, select **End Program**. The current program will be completed and the collected data will be saved.
3. Abort the Run: To stop the run, click on **Abort Run**. *Note* -This will result in loss of all data.
4. **Color compensation** can be turned on or off during a run. Note- color compensation becomes important if you are doing multiplexed assays, but is unnecessary for an experiment where only SYBR Green is used as the fluorescent reporter molecule.



## 5) Appendix A: General Information

### 5.1 ORDERING INFORMATION FOR SYBR GREEN ASSAY

#### ● Reagents and Plates Needed for a SYBR Green Assay

Product	Catalog Name	Catalog Number	Description
SYBR Green Master Mix	LightCycler® 480 SYBR Green 1 Master Mix	04379 012 001	5ml tube of 2x Master Mix. Enough for 250 reactions (20µl reaction volume)
96-well plates	LightCycler® 480 Multiwell Plates 96	04 729 692 001	50 plates and sealing foils
384-well plates	LightCycler® 480 Multiwell Plates 384	04 729 749 001	50 plates and sealing foils

\* Use the following customer account number for a 10% discount: **55240146**.

\* Please see the following page for a more complete list of Roche qPCR products.

### 5.2 REACTION SET-UP

#### ● Wet Set-Up for 20µl Reaction

SYBR Green Set-Up		
Reagent	Final Concentration	Volume Per Reaction
Water	0.5µM	1µl
Forward Primer (5uM Stock)	0.5µM	2µl
Reverse Primer (5uM Stock)	0.5µM	2µl
LightCycler 480 SYBR Green 1 Master	1x	10µl
Total		15µl
Add 5µl cDNA for a total volume of 20µl.		

### 5.3 REACTION VOLUMES

#### ● Appropriate Reaction Volumes

- ☒ 10-100µl for 96-well plates
- ☒ 5-20µl for 384-well plates

**5.4 COMPLETE ROCHE qPCR PRODUCT & PRICE LIST**

Customer Account Number: 55240146		
Roche Cat # or Material #	Description	Net Price Per Item
4728904001	TAQ DNA POLYMERASE DNT Pack ** (2500 U)	\$1,130.40
4729749001	LightCycler 480 Multiwell Plate 384 / 5 x 10 plates (includes sealing foils)	\$360.00
4729757001	LightCycler 480 Sealing Foil / 50 foils	\$67.50
4707494001	LightCycler 480 Probes Master / 5 x 1 ml (2 x conc.) (e.g., for 500 reactions of 20 µl fi- nal reaction volume)	\$288.00
4707516001	LightCycler 480 SYBR Green I Master / 5 x 1 ml (2 x conc.) (e.g., for 500 reactions of 20 µl final reaction volume)	\$288.00
4707524001	LightCycler 480 Genotyping Master / 4 x 384 µl (5 x conc.) (e.g., for 384 reactions of 20 µl reaction volume each)	\$252.00
4902343001	LIGHTCYCLER** 480 PROBE MASTER (1X50 ML)	\$2,557.80
4729692001	LightCycler 480 Multiwell Plate 96 / 5 x 10 plates (includes sealing foils)	\$324.00
4707524001	LightCycler 480 Genotyping Master / 4 x 384 µl (5 x conc.) (e.g., for 384 reactions of 20 µl reaction volume each)	\$252.00
4710924001	LightCycler 480 Control Kit / 1 kit [3 instru- ment demo runs per application (gene quantification and genotyping)]	\$90.00