Technical information

QF 24 V1
CV6 2020

SARS-CoV-2 RT-PCR Detection Kit

Catalog #: COV-2-RTPCR		
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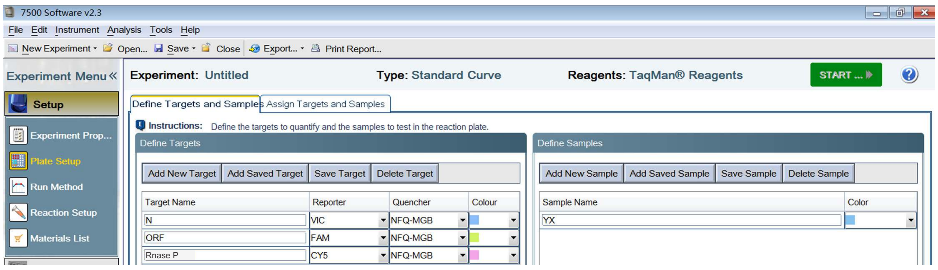
Recommended Sample Type

The SARS-CoV-2 RT-PCR Detection Kit is used for the detection of SARS-CoV-2 viral RNA purified from various clinical samples. The recommended RNA samples are to be extracted from nasopharyngeal, or oropharyngeal swabs.

ABI7500 Setup

1. ABI 7500 Setup for Signal Collection:



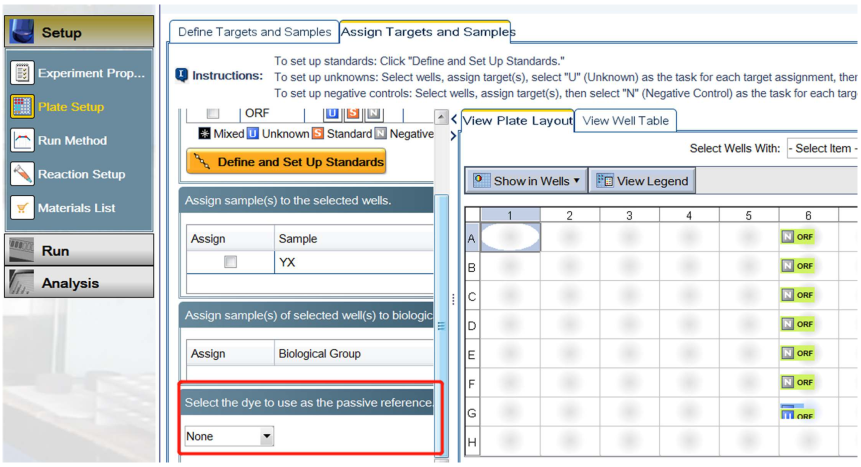
Experiment: Untitled Type: Standard Curve Reagents: TaqMan® Reagents

Instructions: Define the targets to quantify and the samples to test in the reaction plate.

Target Name	Reporter	Quencher	Colour
N	VIC	NFQ-MGB	🟦
ORF	FAM	NFQ-MGB	🟩
Rnase P	CY5	NFQ-MGB	🟪

Sample Name	Color
YX	🟦

2. No Passive Reference Dye Required:



Instructions: To set up standards: Click "Define and Set Up Standards."
To set up unknowns: Select wells, assign target(s), select "U" (Unknown) as the task for each target assignment, ther
To set up negative controls: Select wells, assign target(s), then select "N" (Negative Control) as the task for each targ

Assign sample(s) to the selected wells.

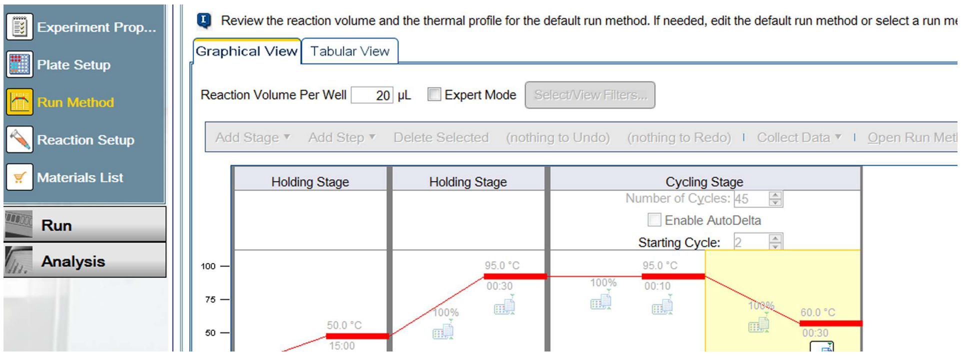
Assign	Sample
☐	YX

Assign sample(s) of selected well(s) to biologic

Assign	Biological Group

Select the dye to use as the passive reference.
None

3. Cycling Protocol:

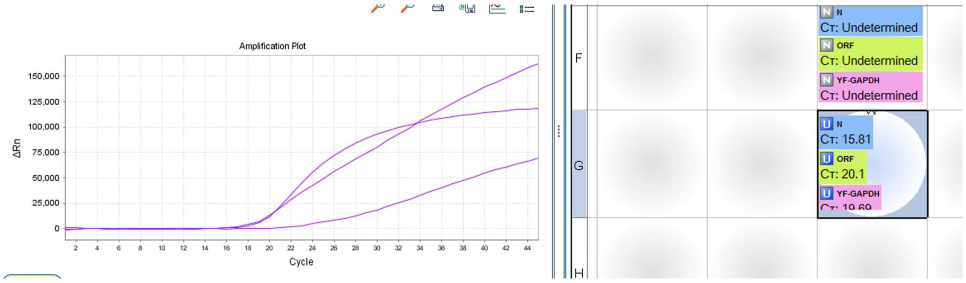


Review the reaction volume and the thermal profile for the default run method. If needed, edit the default run method or select a run me

Reaction Volume Per Well 20 µL ☐ Expert Mode

Number of Cycles: 45
☐ Enable AutoDelta
Starting Cycle: 2

4. Typical Results:



F:
N N CT: Undetermined
N ORF CT: Undetermined
N YF-GAPDH CT: Undetermined

G:
U N CT: 15.81
U ORF CT: 20.1
U YF-GAPDH CT: 19.69

5. Additional Information:

Do we need to select Rox as a passive reference dye or should that be none?

– No passive reference required.

Do you have DMSO in your reaction mix?

– No.

Why does your kit recommend 45 cycles?

– Some positive samples show at Ct from 37-40, 45 cycles is for sufficient amplication.

6. Limitations of Use:

False negatives may be caused by:

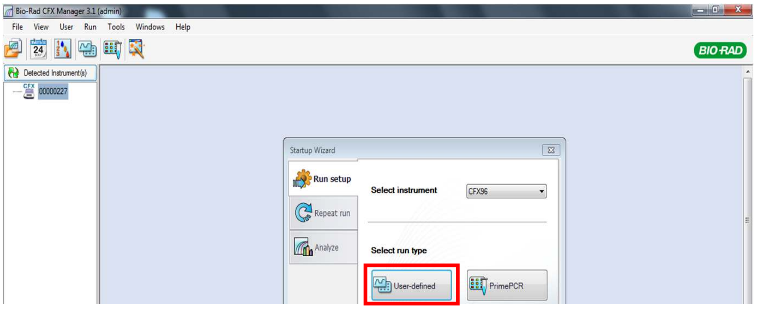
- Unsuitable collection, handling and/or storage of samples.
- Sample outside of viraemic phase.
- Failure to follow handbook procedures.
- Use of unauthorised extraction kit or PCR platform.

False positives may be caused by:

- Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template
- Unsuitable handling of amplified product.

BioRad CFX96 Setup

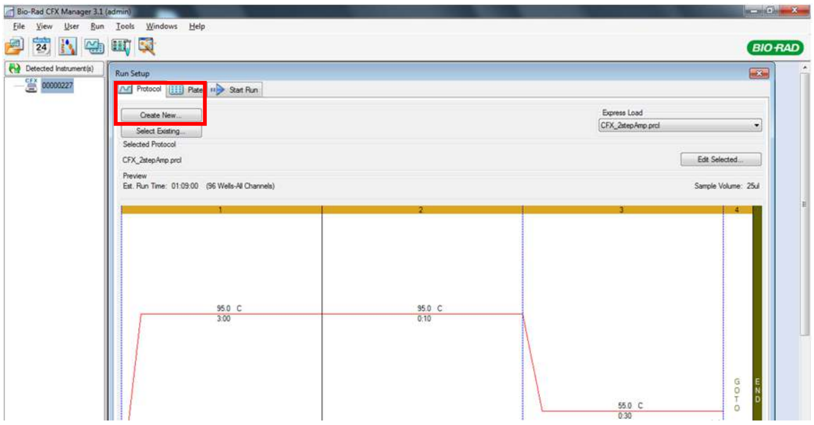
1. Open Bio-Rad CFX Manager Software and select "User-Defined" Run Type.



Select instrument CFX96

Select run type

2. Under Run Setup > Protocol, select "Create New…" to create a new run protocol.

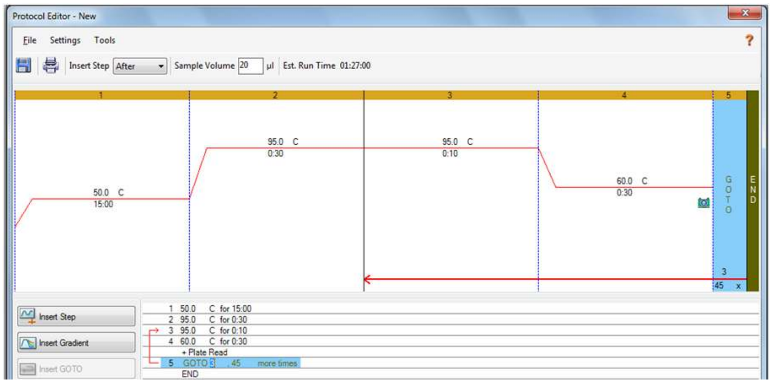


Express Load CFX_2stepAmp.prcl

Selected Protocol CFX_2stepAmp.prcl

Preview
Est. Run Time: 01:09:00 (96 Wells-All Channels)
Sample Volume: 25ul

3. Create Cycling Protocol according to the datasheet. Click “OK”.



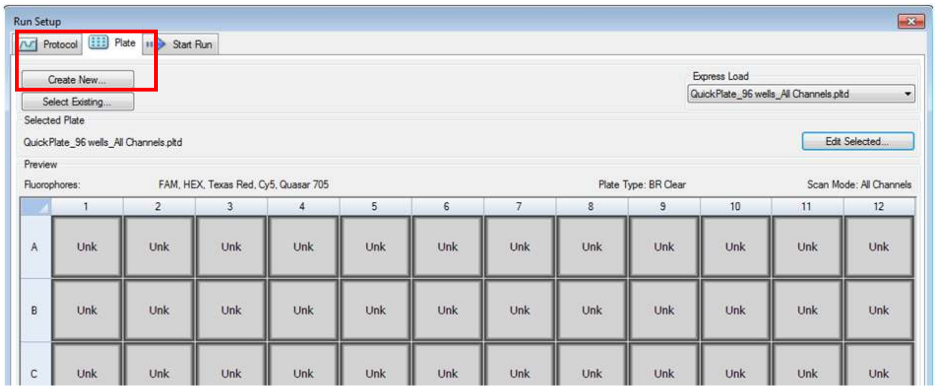
Insert Step: After

Sample Volume: 20 µl

Est. Run Time: 01:27:00

```
1  50.0   C  for 15:00
2  95.0   C  for 0:30
3  95.0   C  for 0:10
4  60.0   C  for 0:30
      + Plate Read
5  GOTO 3  , 45   more times
   END
```

4. Under “Run Setup”, “Plate”, select “Create New…” to create a new Plate set up.



Express Load: QuickPlate_96 wells_All Channels.pltd

Selected Plate: QuickPlate_96 wells_All Channels.pltd

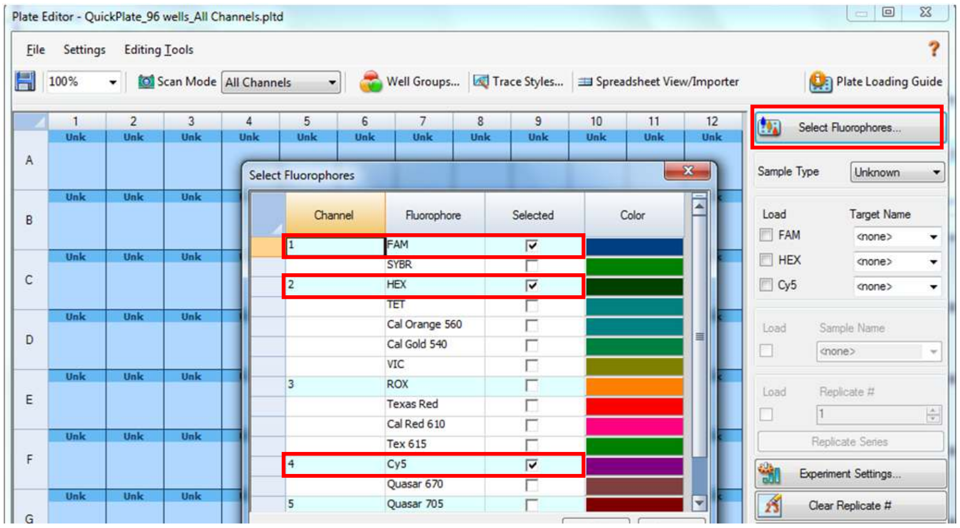
Fluorophores: FAM, HEX, Texas Red, Cy5, Quasar 705

Plate Type: BR Clear

Scan Mode: All Channels

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
B	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
C	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk

5. Use the “Select Fluorophores” Option to define channels: HEX (VIC), FAM and Cy5. Click “OK”.



Zoom: 100%

Scan Mode: All Channels

Channel	Fluorophore	Selected	Color
1	FAM	☑	🟦
	SYBR	☐	🟩
2	HEX	☑	🟩
	TET	☐	🟩
	Cal Orange 560	☐	🟩
	Cal Gold 540	☐	🟩
	VIC	☐	🟨
3	ROX	☐	🟧
	Texas Red	☐	🟥
	Cal Red 610	☐	🟥
	Tex 615	☐	🟩
4	Cy5	☑	🟪
	Quasar 670	☐	🟥
5	Quasar 705	☐	🟥

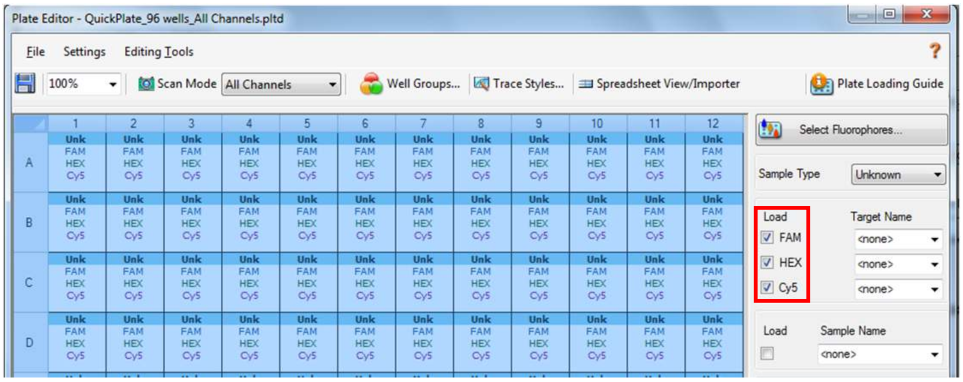
Sample Type: Unknown

Load	Target Name
☐ FAM	<none>
☐ HEX	<none>
☐ Cy5	<none>

Load	Sample Name
☐	<none>

Load	Replicate #
☐	1

6. Apply the three channels to all test wells by selecting the entire plate and checking “Load” for FAM, HEX and Cy5.



Zoom: 100%

Scan Mode: All Channels

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5
B	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5
C	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5
D	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5	Unk FAM HEX Cy5

Sample Type: Unknown

Load	Target Name
☑ FAM	<none>
☑ HEX	<none>
☑ Cy5	<none>

Load	Sample Name
☐	<none>

7. Under Run Setup > Start Run, Click on “Start Run” to start the run.

Run Setup

Express Load: QuickPlate_96 wells_All Channels.pltd

Selected Plate: QuickPlate_96 wells_All Channels.pltd

Preview

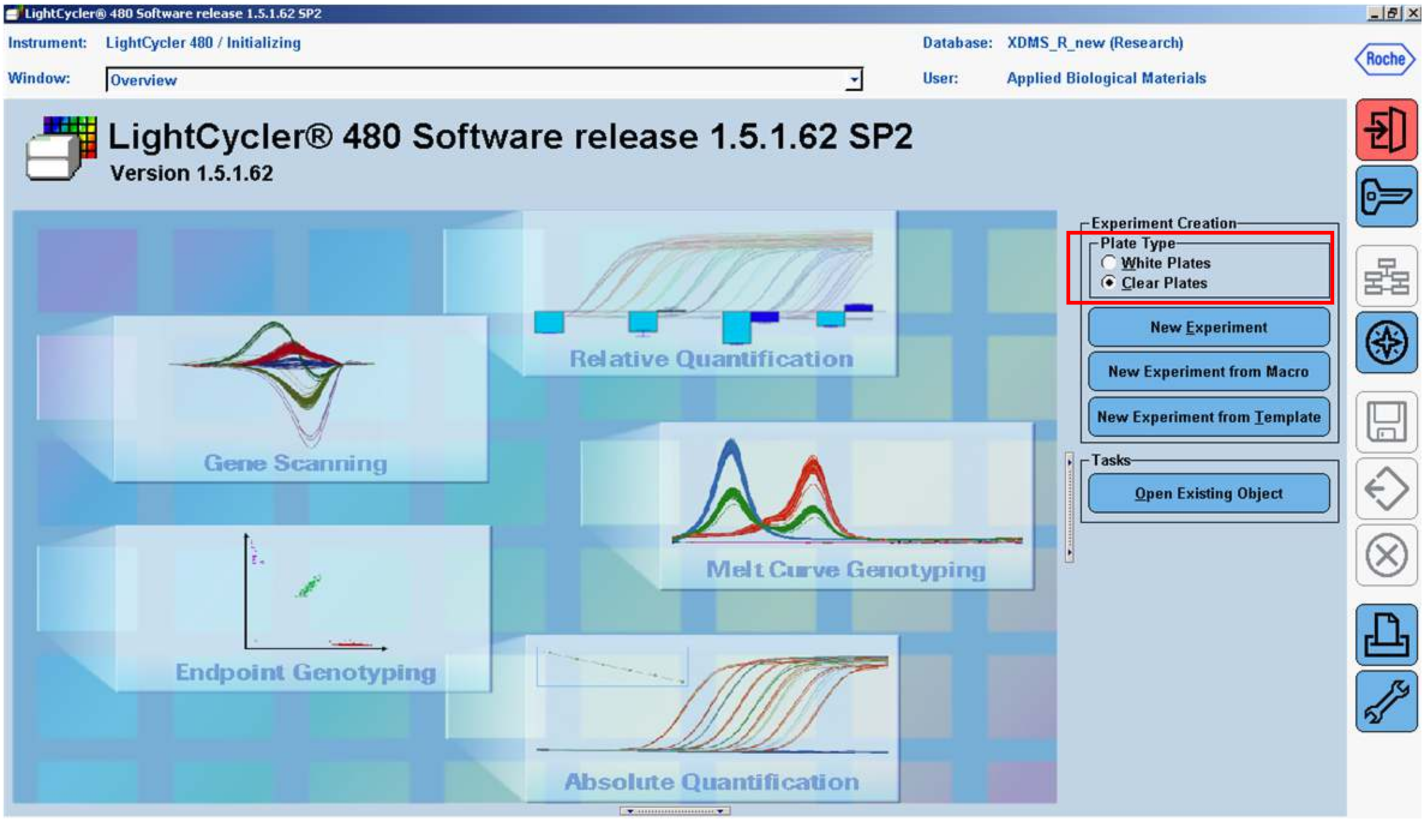
Fluorophores: FAM, HEX, Texas Red, Cy5, Quasar 705

Plate Type: BR Clear

Scan Mode: All Channels

Roche Lightcycler 480 II Setup

1. Open Roche LightCycler 480 Software and select the correct “Plate Type” you are using.



LightCycler® 480 Software release 1.5.1.62 SP2

Instrument: LightCycler 480 / Initializing

Database: XDMS_R_new (Research)

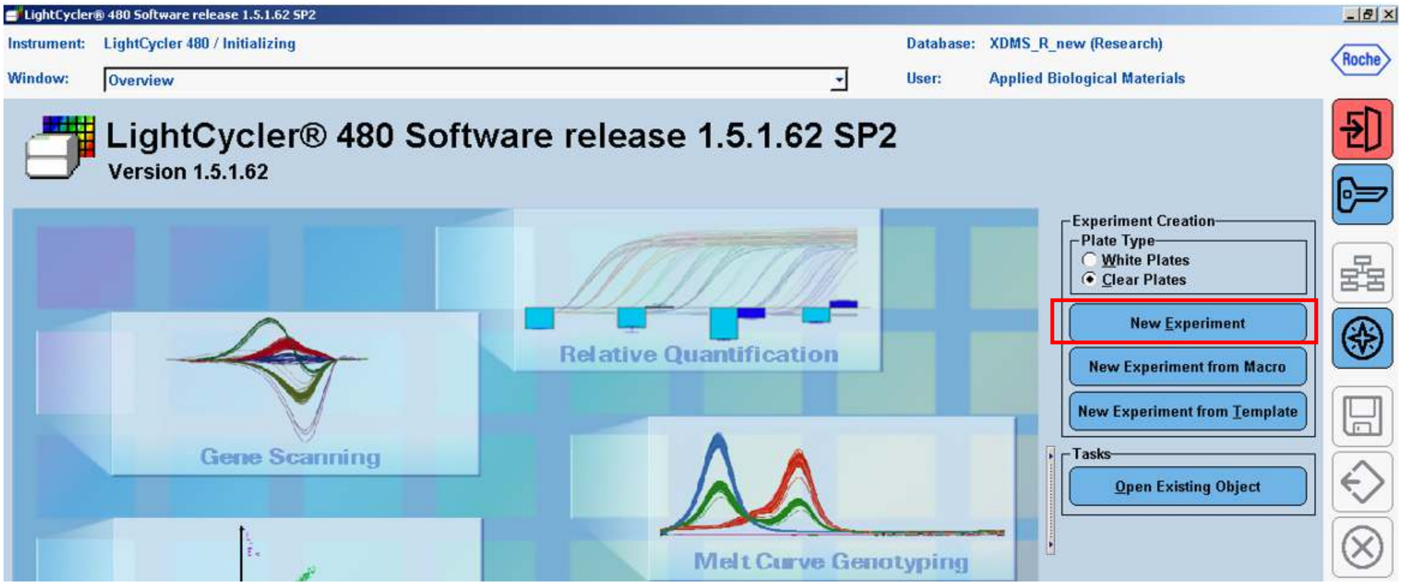
Window: Overview

User: Applied Biological Materials

LightCycler® 480 Software release 1.5.1.62 SP2
Version 1.5.1.62

Experiment Creation
Plate Type
○ White Plates
● Clear Plates

2. Create a New Experiment.



LightCycler® 480 Software release 1.5.1.62 SP2

Instrument: LightCycler 480 / Initializing

Database: XDMS_R_new (Research)

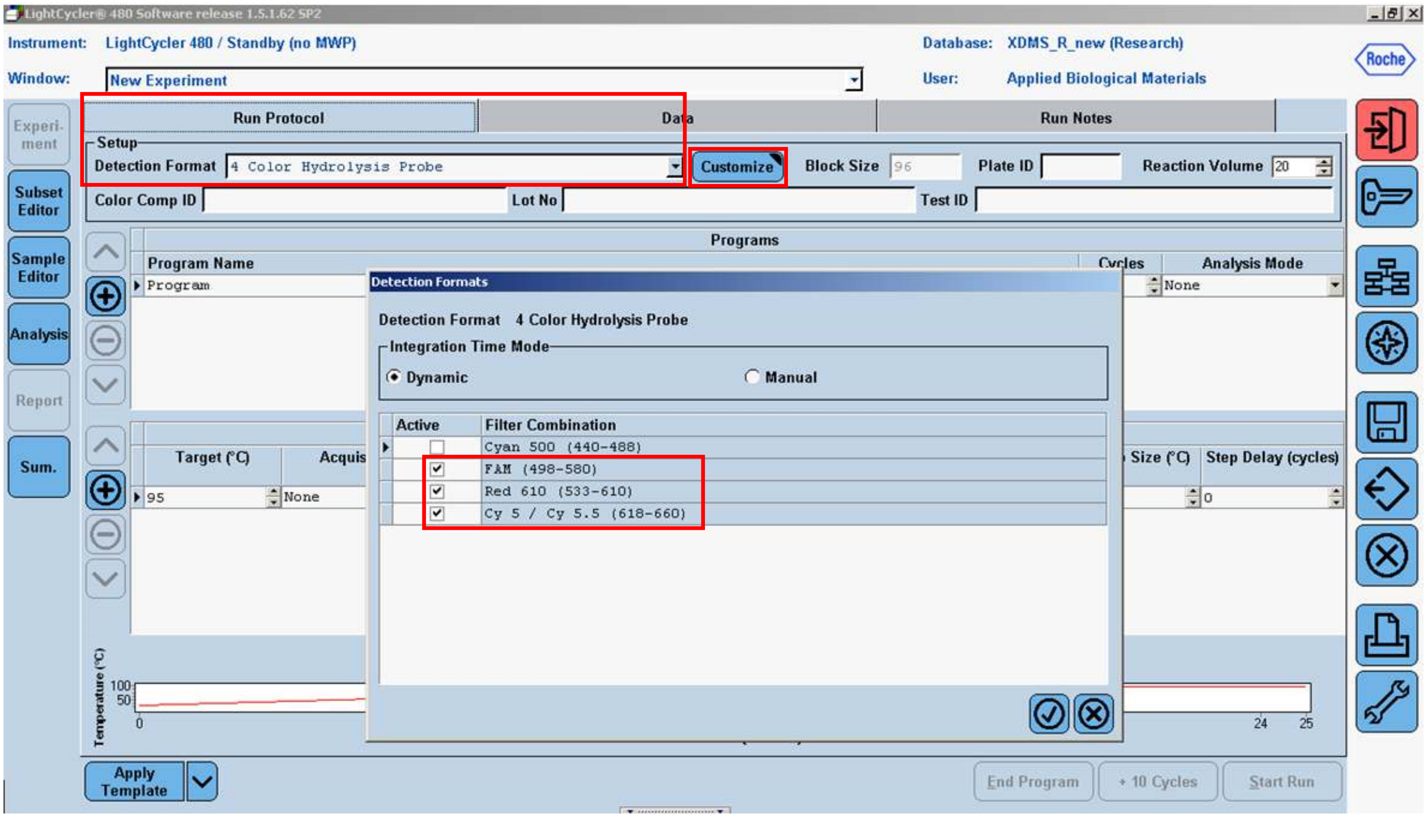
Window: Overview

User: Applied Biological Materials

LightCycler® 480 Software release 1.5.1.62 SP2
Version 1.5.1.62

Experiment Creation
Plate Type
○ White Plates
● Clear Plates

3. Under “Run Protocol” > “Set Up”, select “4 Color Hydrolysis Probe” as the Detection Format. Then click on “Customize”. Check the channels for FAM, Red 610 (HEX) and Cy5. Click OK (✔).



Instrument: LightCycler 480 / Standby (no MWP)

Database: XDMS_R_new (Research)

Window: New Experiment

User: Applied Biological Materials

Setup

Detection Format: 4 Color Hydrolysis Probe

Block Size: 96

Plate ID:

Reaction Volume: 20

Color Comp ID:

Lot No:

Test ID:

Program Name	Cycles	Analysis Mode
Program		None

Target (°C)	Acquis	Size (°C)	Step Delay (cycles)
95	None		0

Detection Formats

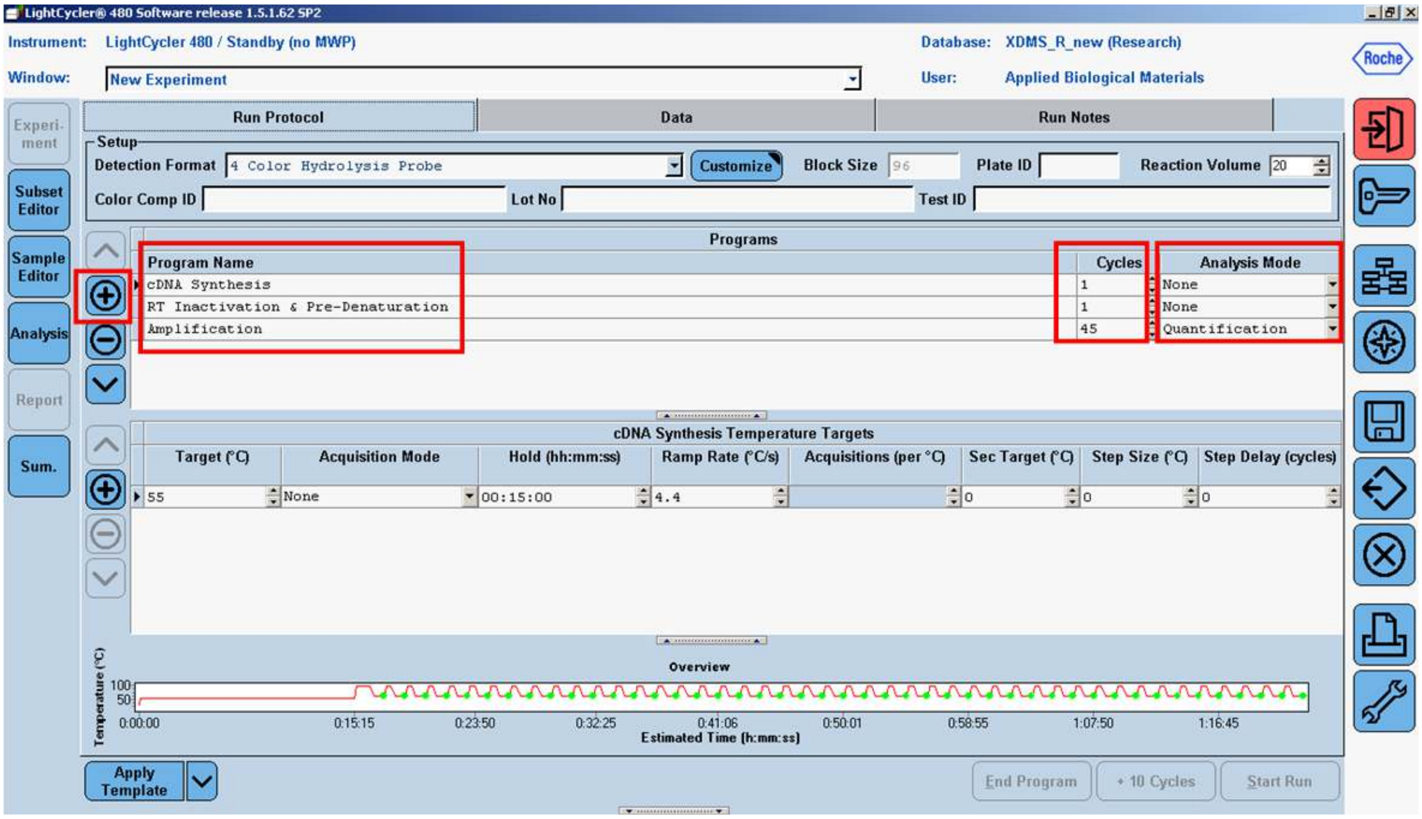
Detection Format: 4 Color Hydrolysis Probe

Integration Time Mode

● Dynamic ○ Manual

Active	Filter Combination
☐	Cyan 500 (440-488)
☑	FAM (498-580)
☑	Red 610 (533-610)
☑	Cy 5 / Cy 5.5 (618-660)

4. Setting up the run conditions. Use the (+) symbol to add 2 additional programs, and name each program accordingly. Enter the number of cycles required for each step. For “Analysis Mode”, make sure “Quantification” is chosen for the last Amplification step.



Instrument: LightCycler 480 / Standby (no MWP)

Database: XDMS_R_new (Research)

Window: New Experiment

User: Applied Biological Materials

Setup

Detection Format: 4 Color Hydrolysis Probe

Block Size: 96

Plate ID:

Reaction Volume: 20

Color Comp ID:

Lot No:

Test ID:

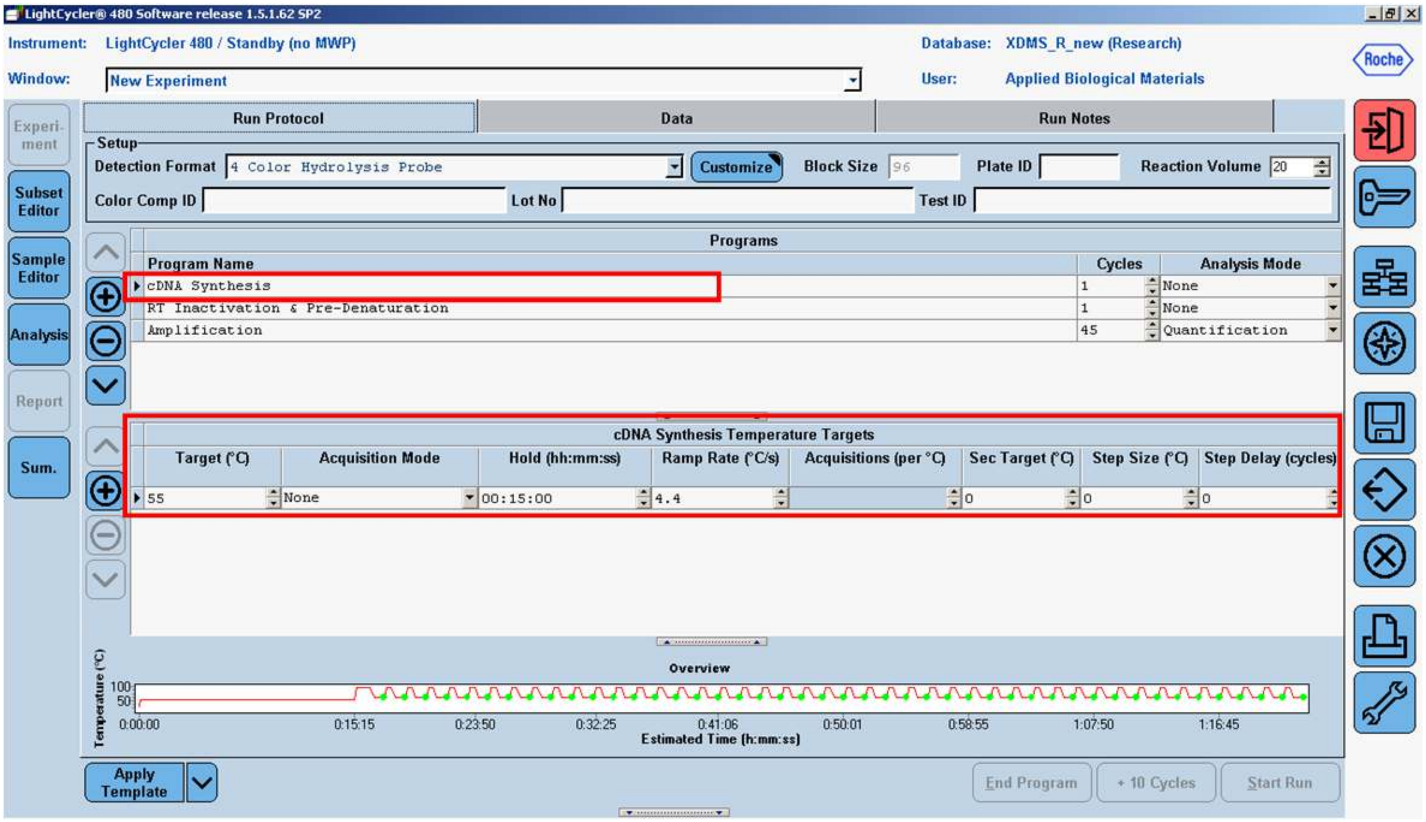
Program Name	Cycles	Analysis Mode
cDNA Synthesis	1	None
RT Inactivation & Pre-Denaturation	1	None
Amplification	45	Quantification

cDNA Synthesis 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)
55	None	00:15:00	4.4		0	0	0

5. For each step, define the temperature and duration.

Click on “cDNA Synthesis” in the “Program Name” section, and then change the setting underneath according to the protocol.



Instrument: LightCycler 480 / Standby (no MWP)
Database: XDMS_R_new (Research)
Window: New Experiment
User: Applied Biological Materials

Setup
Detection Format: 4 Color Hydrolysis Probe
Block Size: 96
Plate ID:
Reaction Volume: 20
Color Comp ID:
Lot No:
Test ID:

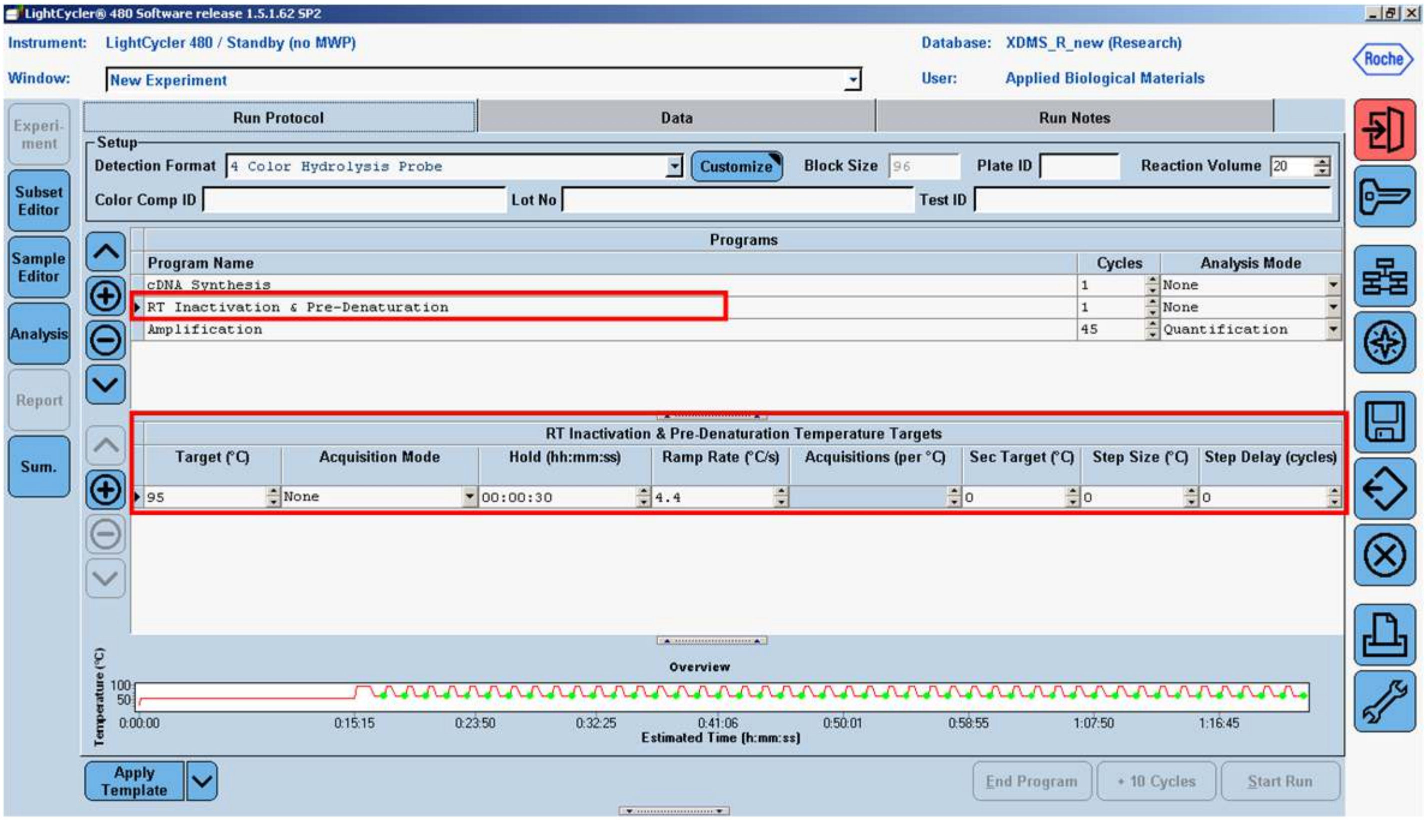
Programs

Program Name	Cycles	Analysis Mode
cDNA Synthesis	1	None
RT Inactivation & Pre-Denaturation	1	None
Amplification	45	Quantification

cDNA Synthesis 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)
55	None	00:15:00	4.4		0	0	0

6.1 Repeat step 5 for the other two programs.



Instrument: LightCycler 480 / Standby (no MWP)
Database: XDMS_R_new (Research)
Window: New Experiment
User: Applied Biological Materials

Setup
Detection Format: 4 Color Hydrolysis Probe
Block Size: 96
Plate ID:
Reaction Volume: 20
Color Comp ID:
Lot No:
Test ID:

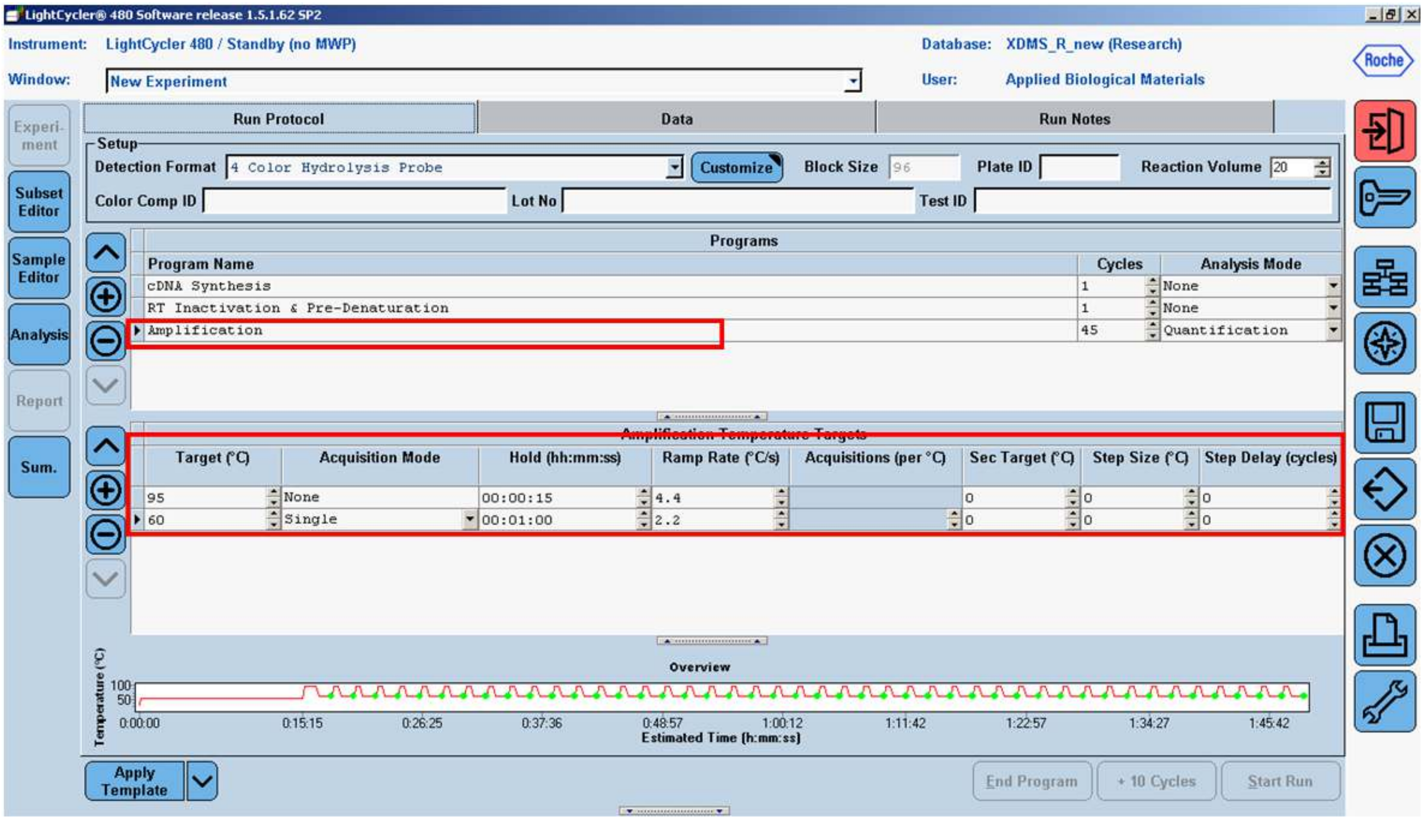
Programs

Program Name	Cycles	Analysis Mode
cDNA Synthesis	1	None
RT Inactivation & Pre-Denaturation	1	None
Amplification	45	Quantification

RT Inactivation & Pre-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	00:00:30	4.4		0	0	0

6.2 For the "Amplification" Program, make sure that the "Acquisition Mode" is set as "Single".



Instrument: LightCycler 480 / Standby (no MWP)

Window: New Experiment

Database: XDMS_R_new (Research)

User: Applied Biological Materials

Setup

Detection Format: 4 Color Hydrolysis Probe

Block Size: 96

Plate ID:

Reaction Volume: 20

Color Comp ID:

Lot No:

Test ID:

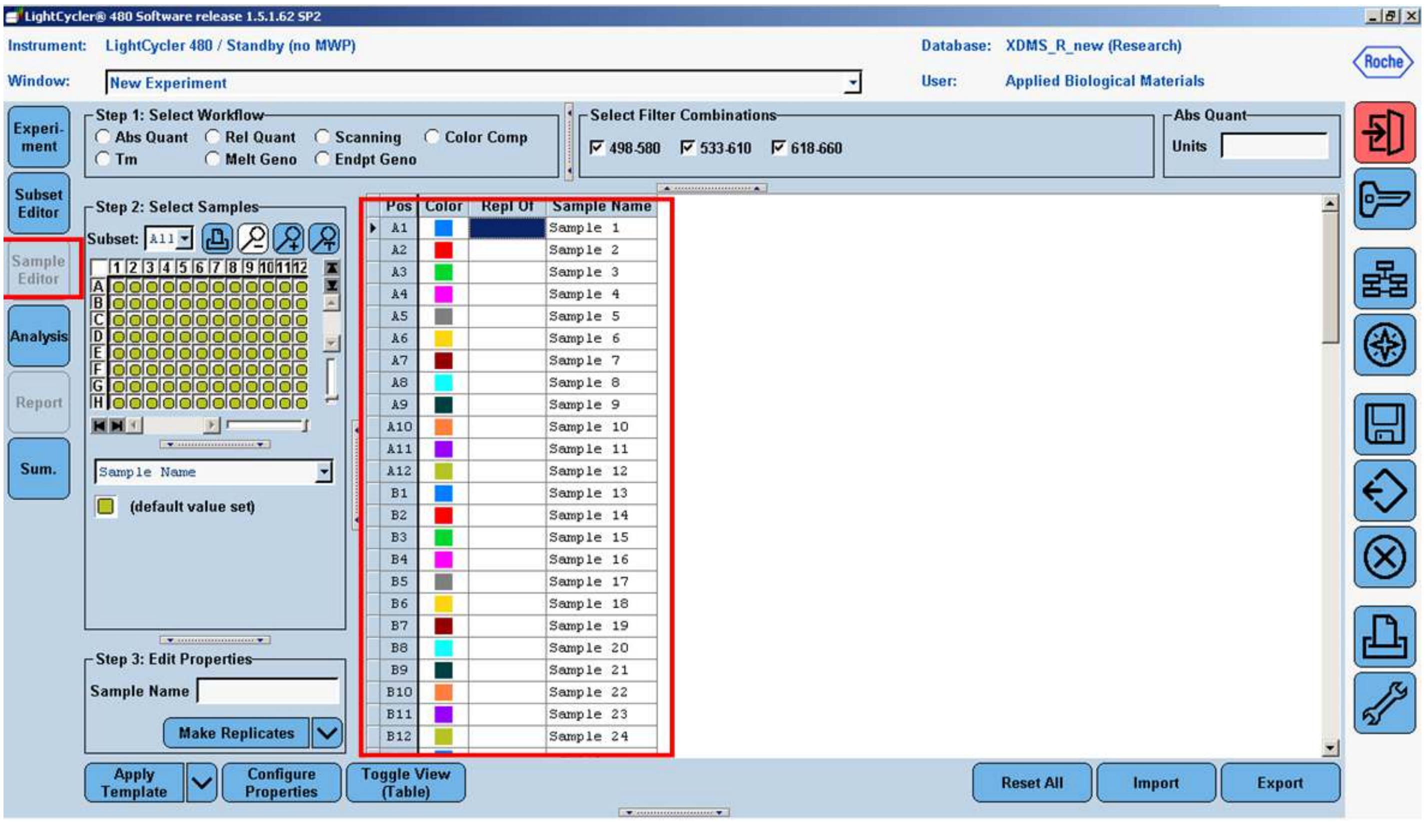
Programs

Program Name	Cycles	Analysis Mode
cDNA Synthesis	1	None
RT Inactivation & Pre-Denaturation	1	None
Amplification	45	Quantification

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	00:00:15	4.4		0	0	0
60	Single	00:01:00	2.2		0	0	0

7. Once the program is set up, you can enter sample names using the "Sample Editor" tab.



Instrument: LightCycler 480 / Standby (no MWP)

Window: New Experiment

Database: XDMS_R_new (Research)

User: Applied Biological Materials

Step 1: Select Workflow

○ Abs Quant ○ Rel Quant ○ Scanning ○ Color Comp
○ Tm ○ Melt Geno ○ Endpt Geno

Select Filter Combinations

☑ 498-580 ☑ 533-610 ☑ 618-660

Abs Quant

Units:

Step 2: Select Samples

Subset: All

Sample Name

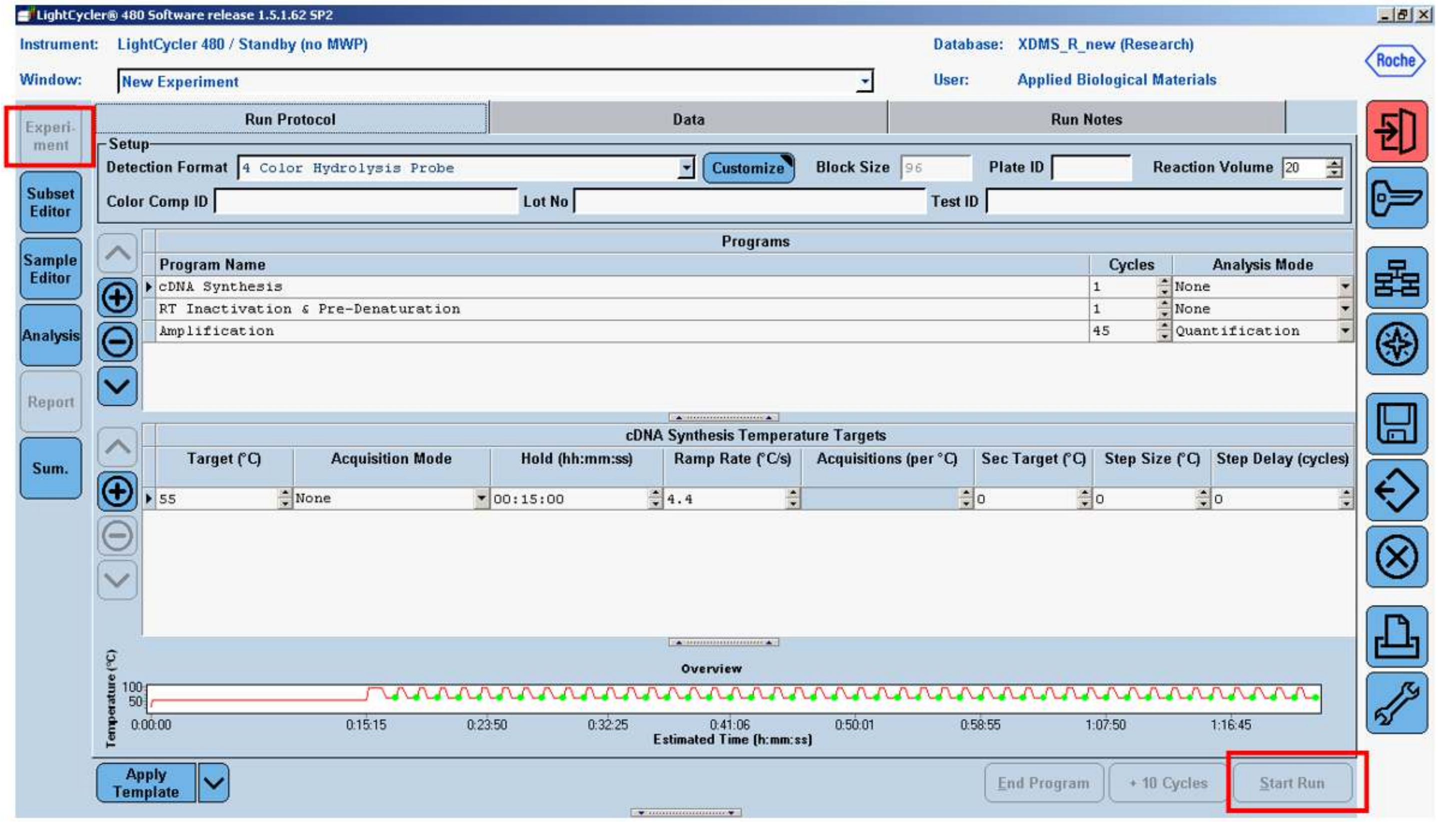
(default value set)

Step 3: Edit Properties

Sample Name:

Pos	Color	Repl Of	Sample Name
A1	🟦		Sample 1
A2	🟥		Sample 2
A3	🟩		Sample 3
A4	🟪		Sample 4
A5	⬛		Sample 5
A6	🟨		Sample 6
A7	🟥		Sample 7
A8	🟦		Sample 8
A9	🟩		Sample 9
A10	🟧		Sample 10
A11	🟪		Sample 11
A12	🟨		Sample 12
B1	🟦		Sample 13
B2	🟥		Sample 14
B3	🟩		Sample 15
B4	🟪		Sample 16
B5	⬛		Sample 17
B6	🟨		Sample 18
B7	🟥		Sample 19
B8	🟦		Sample 20
B9	🟩		Sample 21
B10	🟧		Sample 22
B11	🟪		Sample 23
B12	🟨		Sample 24

8. To run the program, go back to the “Experiment” Tab and click “Start Run”.



Instrument: LightCycler 480 / Standby (no MWP)

Database: XDMS_R_new (Research)

Window: New Experiment

User: Applied Biological Materials

Setup

Detection Format: 4 Color Hydrolysis Probe

Block Size: 96

Plate ID:

Reaction Volume: 20

Color Comp ID:

Lot No:

Test ID:

Programs

Program Name	Cycles	Analysis Mode
cDNA Synthesis	1	None
RT Inactivation & Pre-Denaturation	1	None
Amplification	45	Quantification

cDNA Synthesis 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)
55	None	00:15:00	4.4		0	0	0