RIDA®GENE Color Compensation Kit II

Art. No.: PG0002
3 reactions

-20 °C

R-Biopharm AG, An der neuen Bergstraße 17, D-64297 Darmstadt, Germany
Tel.: +49 (0) 61 51 81 02-0 / Telefax: +49 (0) 61 51 81 02-20

1. Intended use

RIDA®GENE Color Compensation Kit II is intended for generating a Color Compensation File for duplex and triplex real-time PCR experiments on the LightCycler ® 1.5 and 2.0. The generated Color Compensation File can be applied to analyze multiplex real-time PCR experiments of RIDA®Gene real-time PCR kits on the LightCycler® 1.5 and 2.0.

2. Explanation of the test

In a multiplex real-time PCR, the wavelengths of light emitted by the reporter dyes may overlap, causing one channel to pick up signals (crosstalk) from a dye measured by another channel. This crosstalk of fluorescence signal can result in incorrect data unless a correction is made by using a Color Compensation File. Color compensation is used to subtract fluorescence crosstalk from a reporter dye into inappropriate channels outside of its dominant emission channel.

3. Kit components

Tab.1: Reagents provided (Reagents provided in the kit are sufficient for 3 Color Compensation experiments)

Kit Code	Reagent	Volume	Lid color
1	Blank	1x 80 µl	white
2	Dye 1	1x 80 µl	green
3	Dye 2	1x 80 µl	yellow
4	Dye 3	1x 80 µl	red

4. Storage instructions

- Protect RIDA®GENE Color Compensation Kit II from light and store at -20 °C.
- RIDA®GENE Color Compensation Kit II can be used until the expiration date printed on the label
- After expiry the quality guarantee is no longer valid.
- Carefully thaw RIDA®GENE Color Compensation Kit II reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- During Color Compensation preparation all the reagents should be stored cold in an appropriate way (2 - 8 °C).

5. Additional equipment and materials required

- LightCyler® 1.5 or 2.0 (Roche)
- Real-time PCR consumables (LightCycler® Capillaries)
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips

6. Precautions for users

- This test must only be performed by laboratory personnel trained in molecular biology methods.
- Strictly follow the working instructions.
- When handling samples, wear disposable gloves. After finishing the test, wash your hands.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- Do not use the kit after the expiration date.

7. Protocol for creating a Color Compensation File on the LightCycler® 1.5

7.1 Preparation of the Color Compensation

For a color compensation experiment it is necessary to pipette 20 µl of each dye and also of the background (blank) into a LightCycler® capillary. Add 1 µl of Dye 1 to the pre-pipetted 20 µl of Dye 3 in LightCycler® capillary 4 (see Tab.2).

Thaw, mix gently and centrifuge briefly the reagents before use. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab.2: Preparation of the Color Compensation

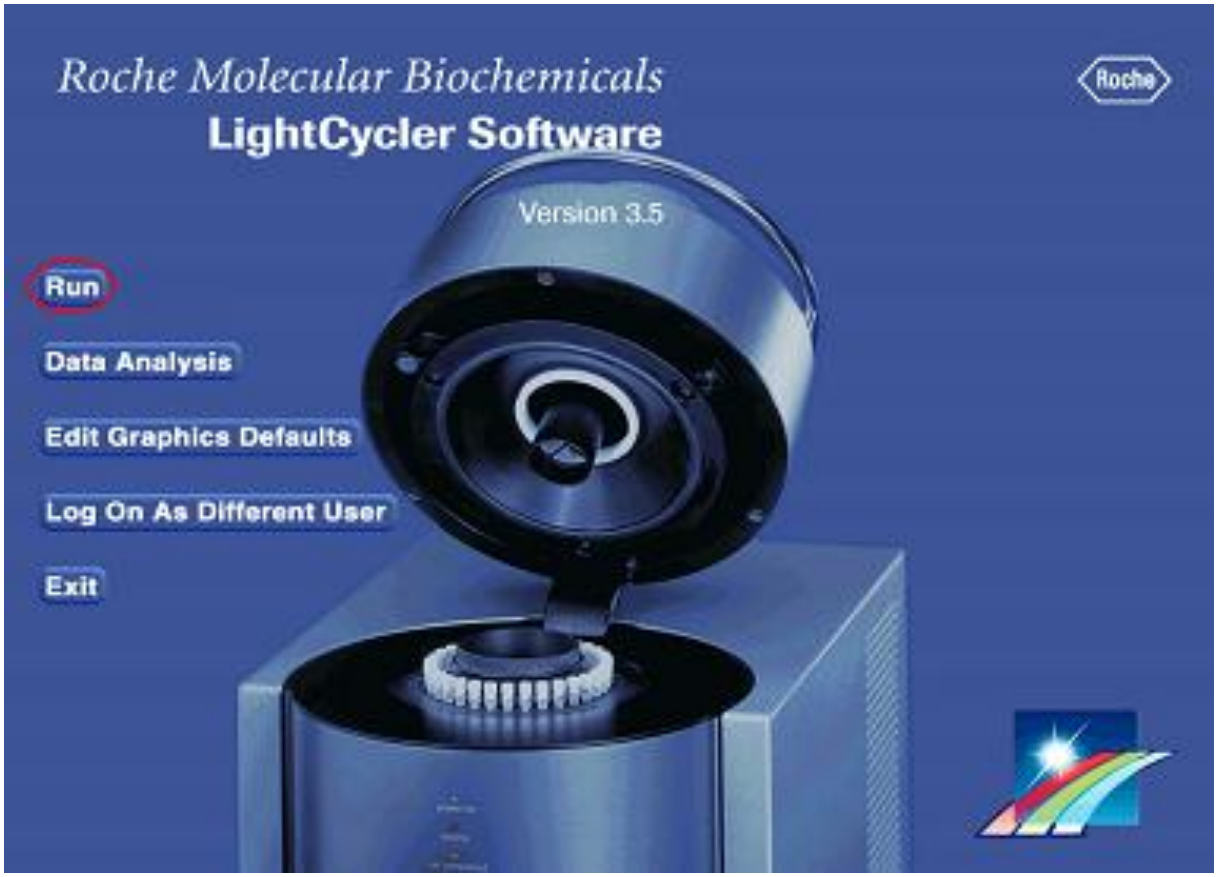
Kit Code	Reagent	Volume per reaction			
		Capillary Position 1	Capillary Position 2	Capillary Position 3	Capillary Position 4
1	Blank	20 µl	-	-	-
2	Dye 1	-	20 µl	-	1 µl
3	Dye 2	-	-	20 µl	-
4	Dye 3	-	-	-	20 µl

Cap and briefly spin down the LightCycler® capillaries after pipetting the reagents.

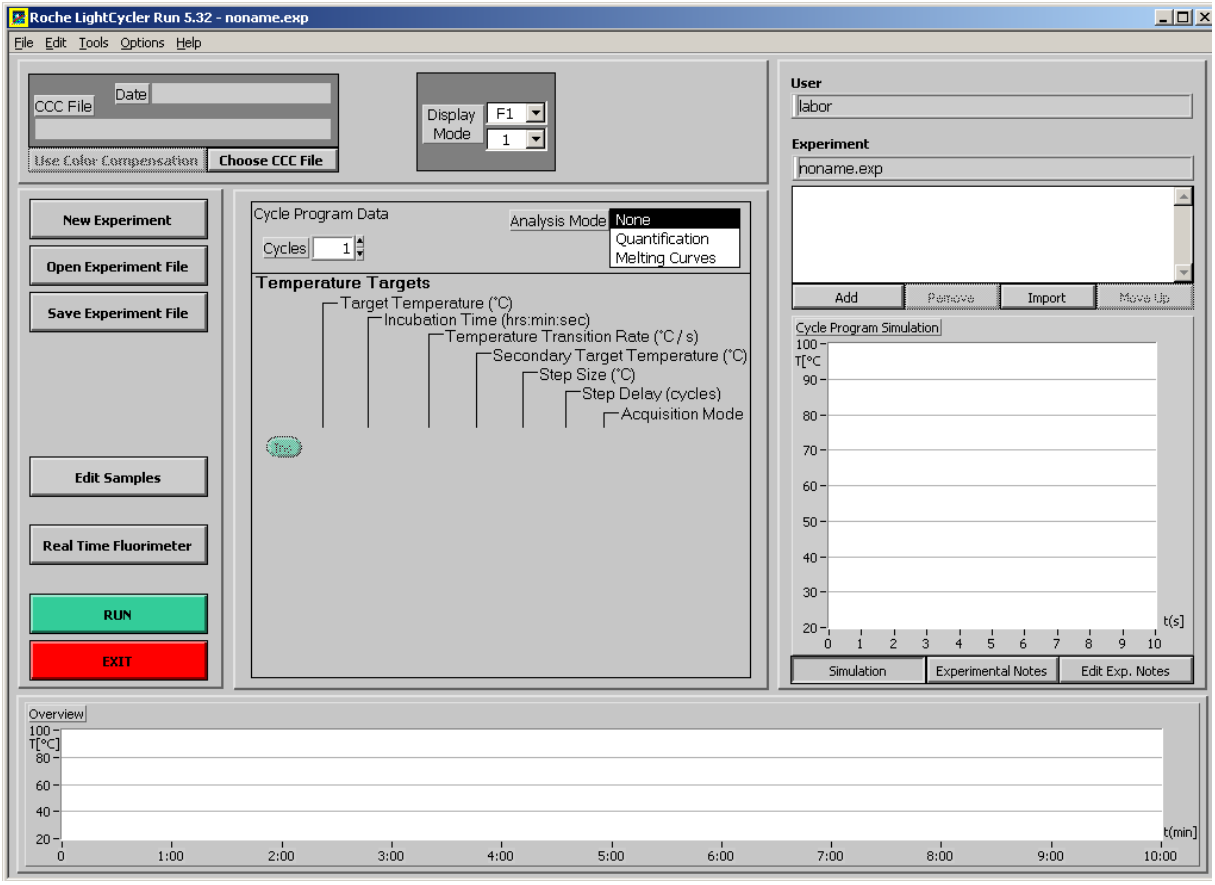
Note: Do not change the order of the LightCycler® capillaries on the LightCycler®-Carousel.

7.2 LightCyler® 1.5 Set-up

1. Open the LightCycler® 1.5 software. Click the “**Run**” button to open a new “**LighCycler Experiment**”.



2. The following window opens.

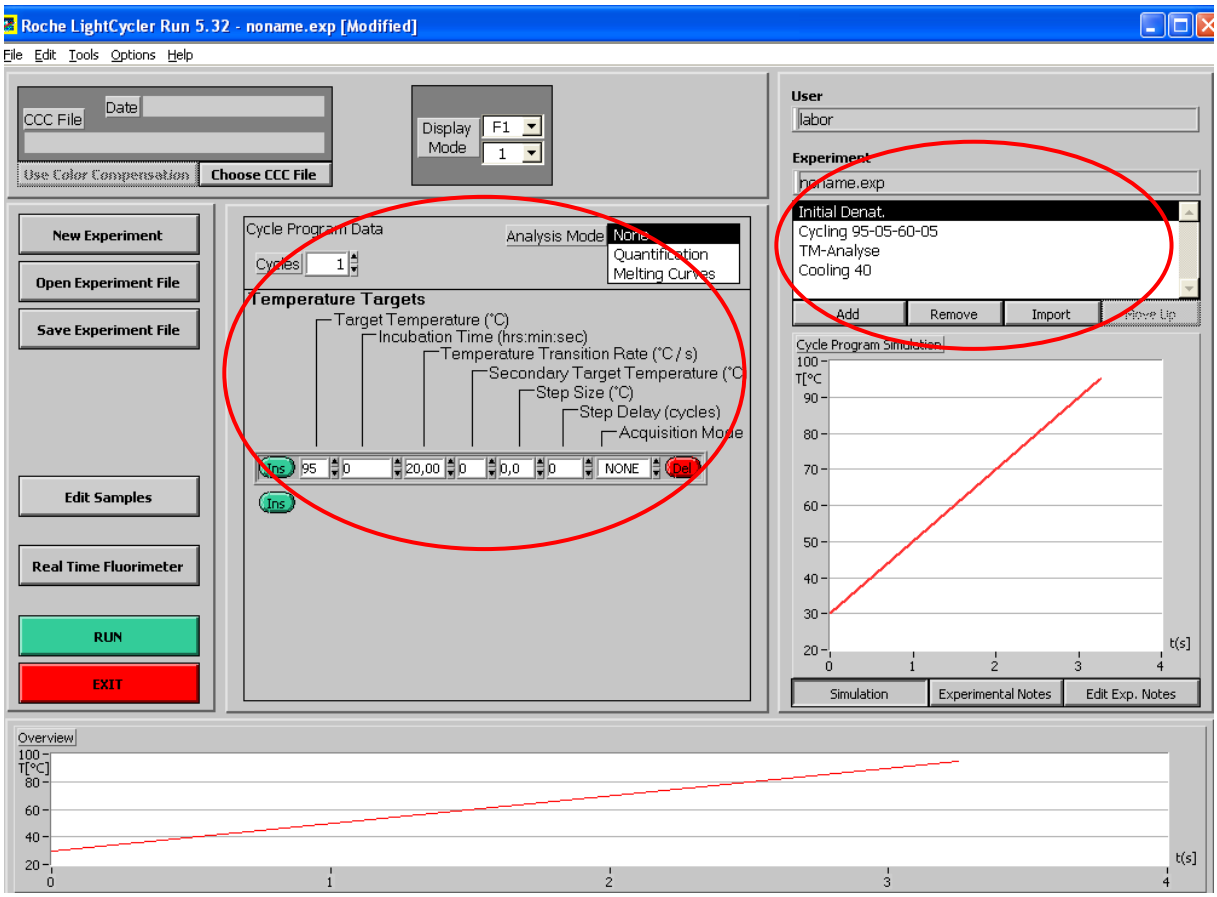


3. Program the LightCycler® according to the real-time PCR profile (see Tab.3). Click the "**Add**" button to program the 4 protocol steps.

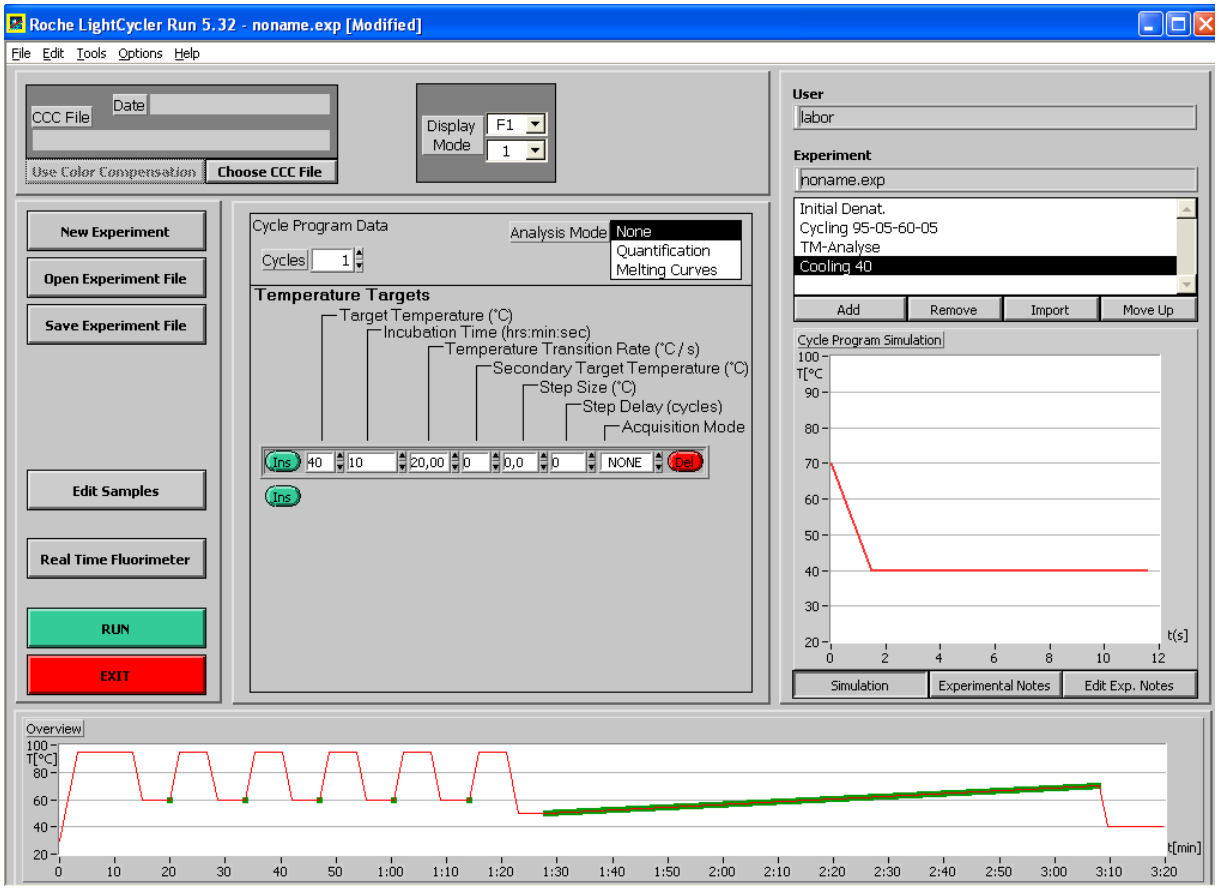
Tab.3: LightCycler® real-time PCR profile

		Temperature targets			
Program	**Cycles / Analysis Mode**	**Target [°C]**	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp rate [°c/s]**
Initial Denat.	1 / none	95	none	00:00:05	20
Cycling	5 / Quantification	95	none	00:00:05	20
		60	single	00:00:05	20
TM-Analyse	1 / Color Compensation	95	none	00:00:05	20
		50	none	00:00:05	20
		70	continuous		0.2 (Acquisitions per °C = 1)
Cooling 40	1 / none	40	none	00:00:10	20

Note: Ensure the correct setting of the number of "**Cycles**" and of the "**Analysis Mode**"

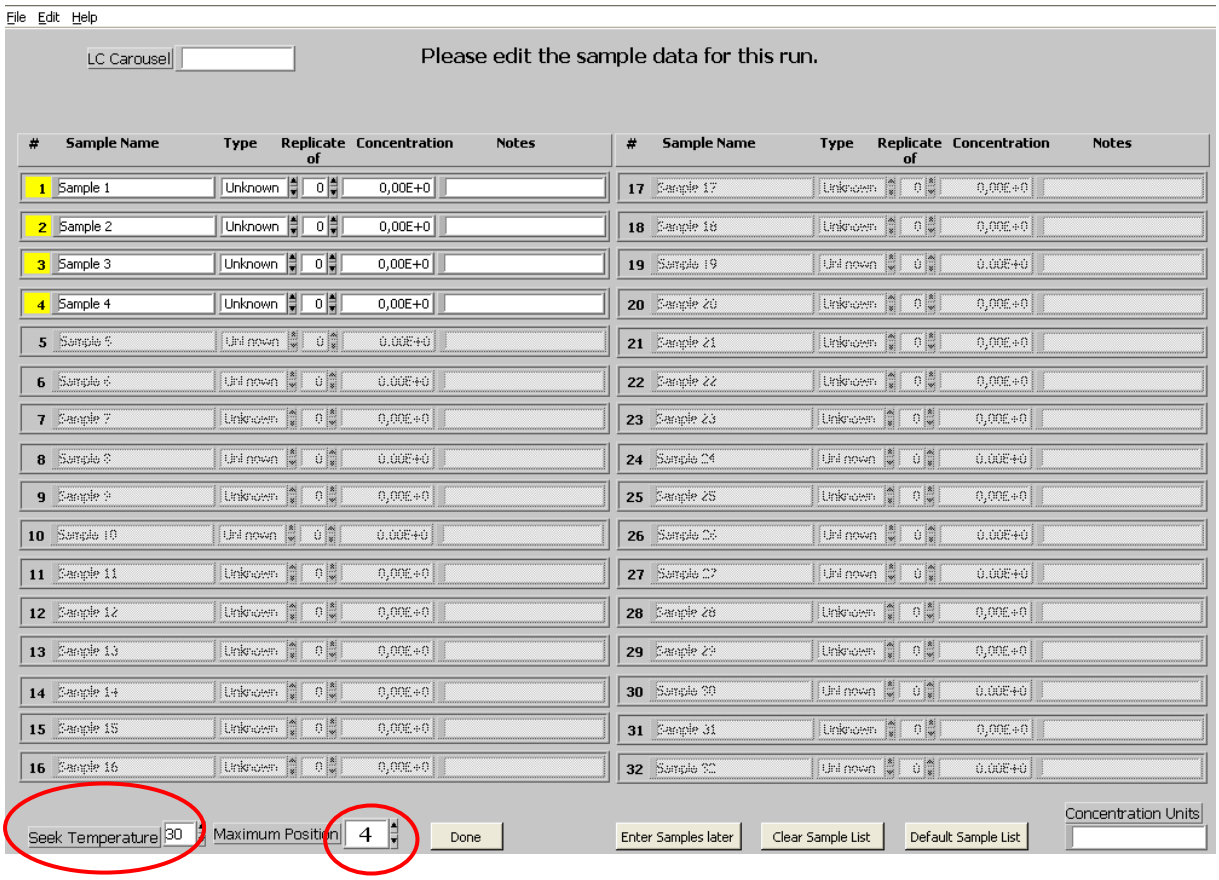


4. The final screen should look like the screenshot below.

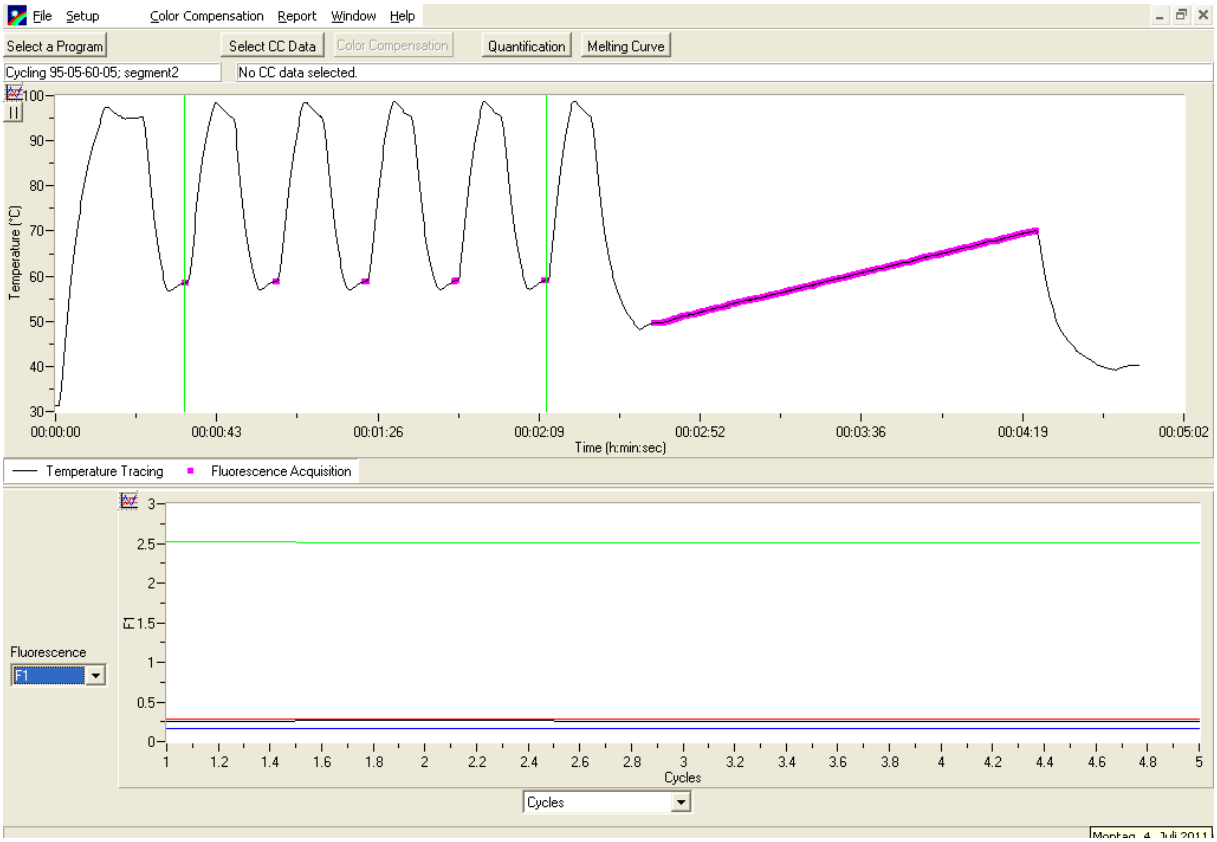


5. Place the LightCycler®-Carousel with the capillaries in the LightCycler® 1.5. Click the “**Run**” button and save the experiment in the proper folder.

The following window will open to program the PCR layout. Enter in the “**Maximum Position**” dialog field the number of capillaries. Click the “**Done**” button to start the experiment.

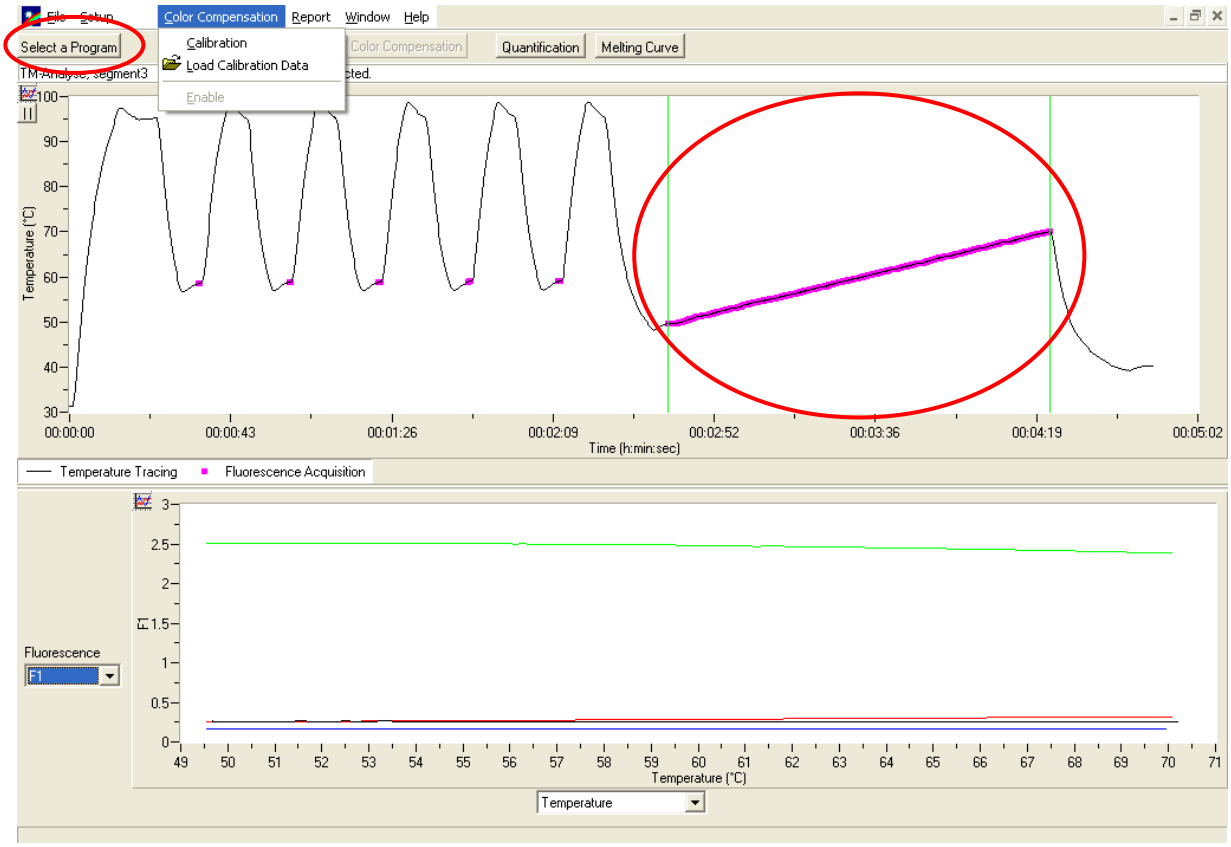


1. After the experiment ends the following window opens.



2. Click “**Select a Program**” button to select segment 3 (TM-Analyse) for Color Compensation.

Ensure that the green cursors are placed around the start and end of the melting curve segment. Then select “**Calibration**” from the Color Compensation pull-down menu.



Save the Color Compensation File. The created Color Compensation File can now be applied for LightCycler® experiments.

To apply Color Compensation for a multiplex PCR experiment click the “**Select CC Data**” button to select and import the appropriate Color Compensation File. Select the appropriate channel (e.g. F1 or F2) and click the “**Quantification**” button to analyze the experiment.

Note: The Color Compensation File is specific for every LightCycler® instrument. A new Color Compensation File has to be created if the LightCyler® instrument is changed or after the optical system has been repaired.

8. Protocol for creating a Color Compensation File on the LightCycler® 2.0

8.1. Preparation of the Color Compensation

For a color compensation experiment it is necessary to pipette 20 µl of each dye and also of the background (blank) into a LightCycler® capillary. (see Tab.4).

Thaw, mix gently and centrifuge briefly the reagent before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab.4: Preparation of the Color Compensation

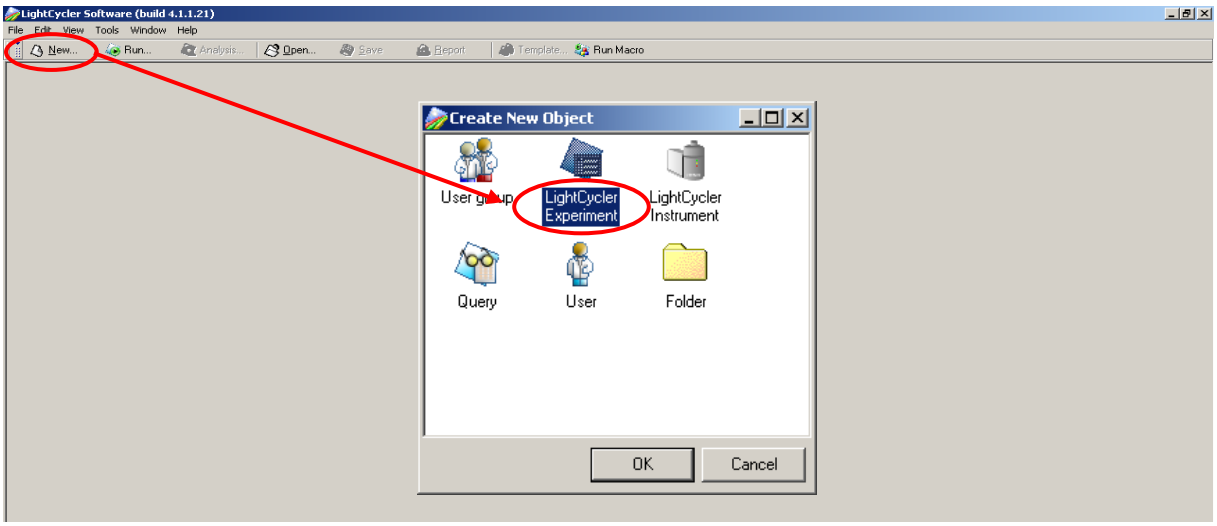
Kit Code	Reagent	Volume per reaction	Capillary position
1	Blank	20 µl	1
2	Dye 1	20 µl	2
3	Dye 2	20 µl	3
4	Dye 3	20 µl	4

Cap and briefly spin down the LightCycler® capillaries after pipetting the reagents.

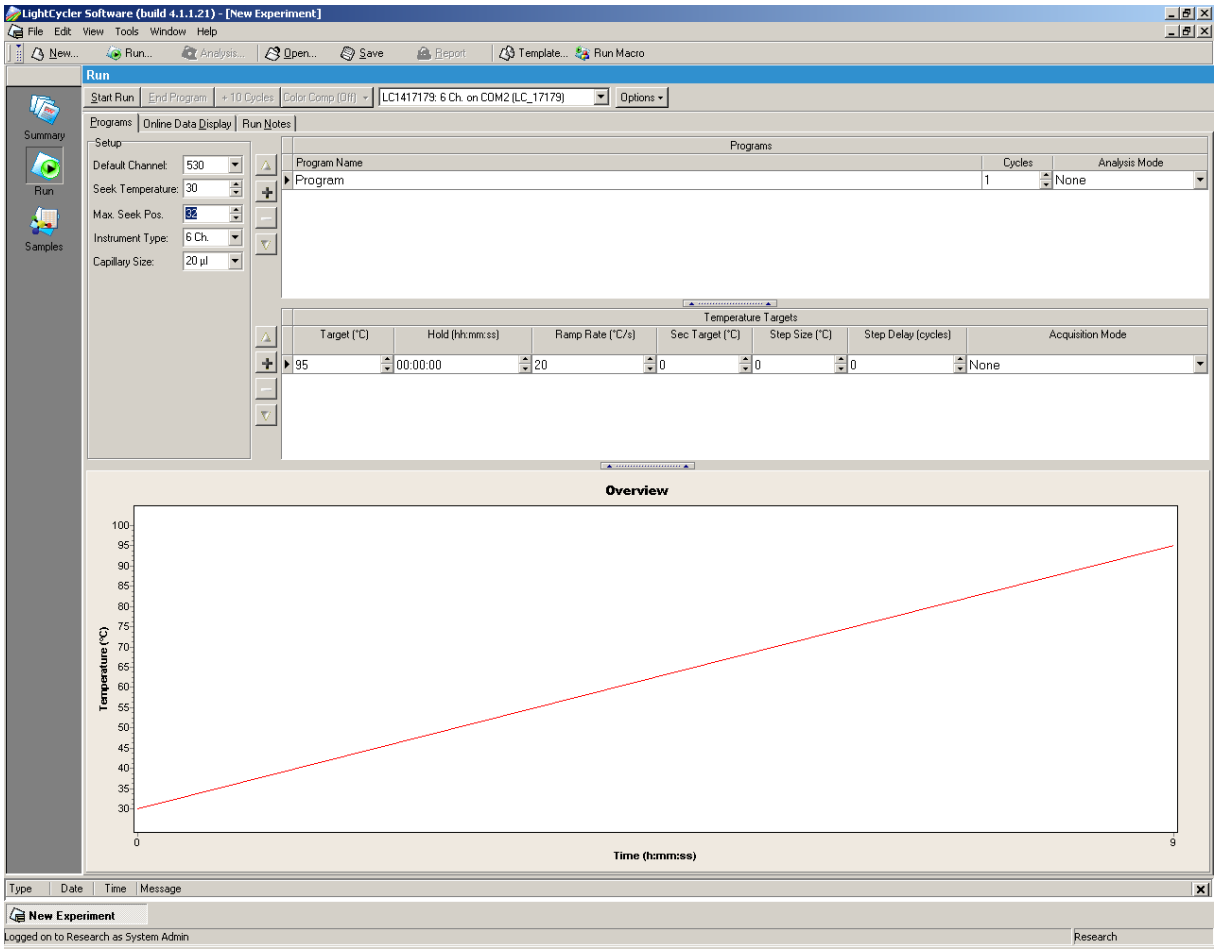
Note: Do not change the order of the LightCycler® capillaries on the LightCycler® Carousel.

8.2 LightCyler® 2.0 Set-up

1. Open the LightCycler® 2.0 software. Click the "**File**" menu, and select "**New**" to open the "**Create New Object**" dialog box. Double-click the "**LightCycler Experiment**" icon to open a new LightCycler experiment file.



2. The following window opens.



3. Program the the LightCycler® according to the Set-up (see Tab. 5) and the protocol steps of the real-time PCR profile (see Tab. 6)

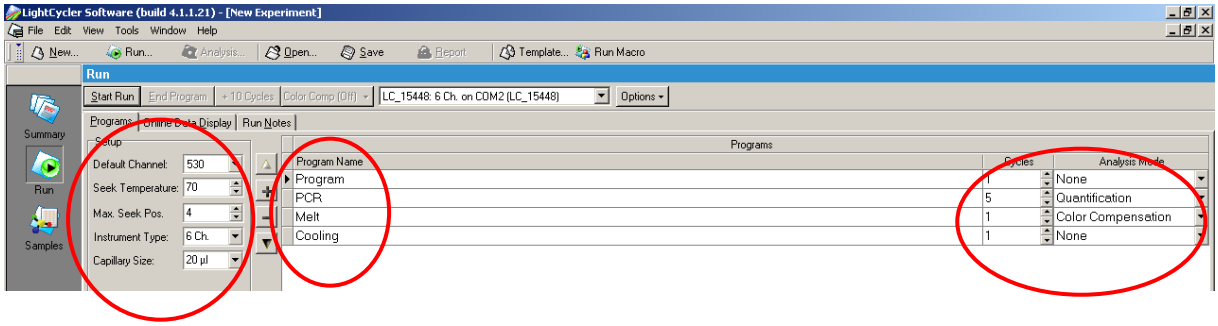
Tab.5: LightCycler® Set-up

Parameter	Setting
Default Channel	530
Seek Temperature	**70 °C**
Max. Seek Pos.	4
Instrument Type	6 Ch.
Capillary Size	20µl

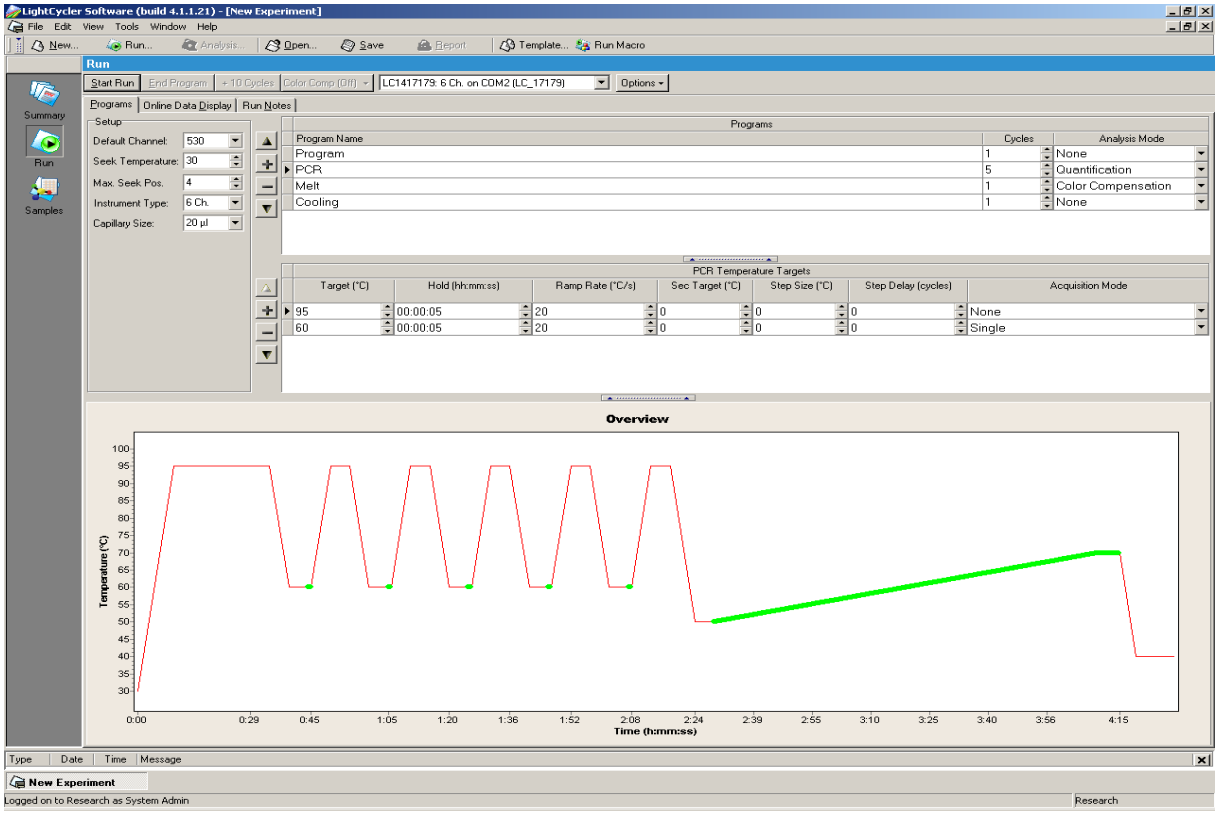
Tab.6: LightCycler® real-time PCR profile

		Temperature targets			
Program	**Cycles / Analysis Mode**	**Target [°C]**	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp rate [°c/s]**
Initial Denat.	1 / none	95	none	00:00:05	20
Cycling	5 / Quantification	95	none	00:00:05	20
		60	single	00:00:05	20
TM-Analyse	1 / Color Compensation	95	none	00:00:05	20
		50	none	00:00:05	20
		70	continuous		0.2 (Acquisitions per °C = 1)
Cooling 40	1 / none	40	none	00:00:10	20

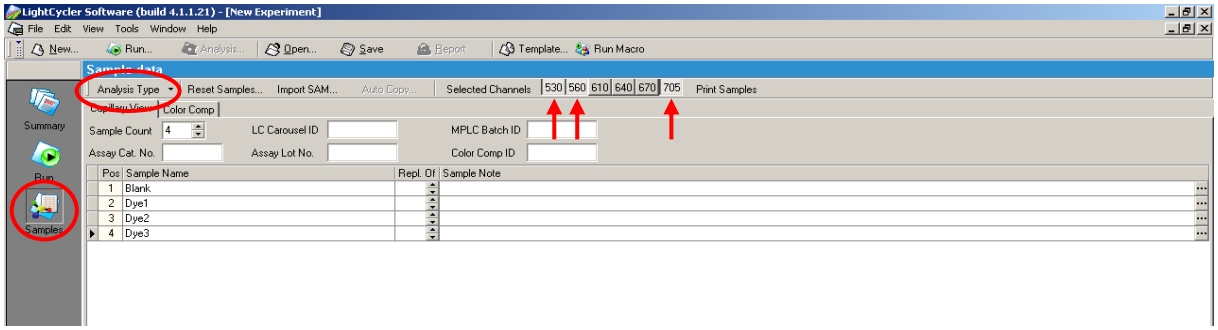
Note: Ensure the correct setting of the number of "**Cycles**" and of the "**Analysis Mode**"



4. The final screen should look like the screenshot below.



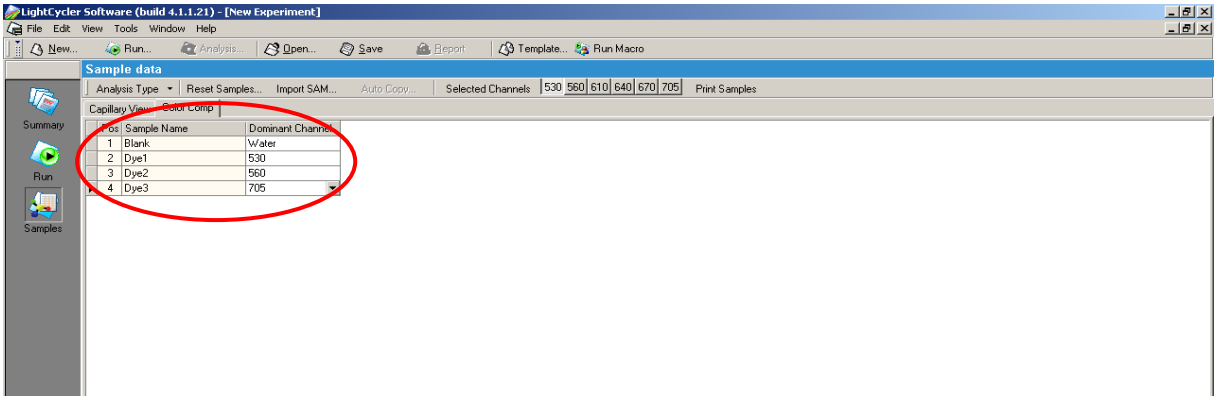
5. Click the "**Samples**" icon on the left-hand side of the window. Then click the "**Analysis Type**" menu, and select "**Color Compensation**".



6. Define in the "**Dominant Channel**" dialog fields the dominant channel for the selected dyes for each position (see Tab.7). Select for the reagent "**Blank**" the dominant channel "**Water**". It is not necessary to specify a "**Sample Name**".

Tab.7: Detection Channel Set-up

Reagent	**Detection Channel**
Blank	Water
Dye1	530
Dye2	560
Dye3	705



Place the LightCycler®-Carousel with the capillaries in the LightCycler® 2.0. Click the "**Run**" icon and save the experiment in the proper folder.

8.3 Evaluation and creation of a Color Compensation File

After the experiment ends, click the "**Analysis**" button to open the "**Create New Analysis**" dialog box. Under "**Other Methods**", select "**Color Compensation**". Then click "**OK**"

In the "**Color Compensation**" window which appears, click the "**Save CC Object**" button to save the Color Compensation File in the "**CCC"** folder. The created Color Compensation File can now be applied for LightCycler® experiments.

To apply Color Compensation for a multiplex PCR experiment open the experiment and click the "**Analysis**" button. Click the "**Color Compensation (Off)**" button, and select the appropriate Color Compensation File. The "**Color Compensation (Off)**" button switches to "**Color Compensation (On)**" to confirm that color compensation is active. The multiplex real-time PCR experiment can now be analyzed.

Note: The Color Compensation File is specific for every LightCycler® instrument. A new Color Compensation File has to be created if the LightCyler® instrument is changed or after the optical system has been repaired.