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# Product Manual

Of

## Q-MRSA Detection Kit

For simultaneous detection of three MRSA markers in one  
real-time PCR reaction

*Note: Research Use Only. Not intended for use in diagnostic procedures.*

For the following products:

Cat. No. 1-300-10010

Cat. No. 1-300-10020

*Note: 'Q-Fusion' and 'Yin-Yang Probes' are trademarks belonging to QuanDx Inc. These products and the methods employed are covered by United States Patent Numbered 7,799,522; EU Patent Numbered 1339732; Japan Patent Numbered 3999653; Australia Patent Numbered 2001296647; China Patent Numbered 01817012.9; India Patent Application Number 1230/DELNP/2005; Brazil Patent Application Number PI0114575-4, and Canada Patent Application Number 2,424,856. QuanDx owns all of these patents.*

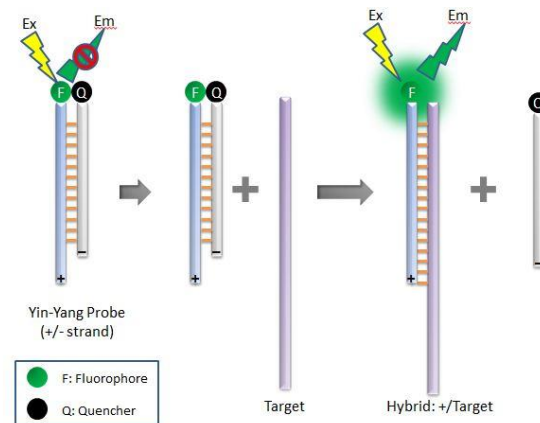
## PURPOSE OF THE TEST

The Q-MRSA Detection Kit is a multiplex real-time PCR system for the detection of Methicillin-Resistant Staphylococcus Aureus (MRSA). This kit detects three MRSA markers all at once by specific fluorescence probes: the *mecA* gene, the *nuc* gene (*S. aureus*-specific marker) and *SCCmec:orfX* junction (the integration site of the SCCmec cassette into the *S. aureus* chromosome). Rapid results can be obtained within 2 hours. The MRSA Screening system provides a sensitive, specific, reliable and cost-effective tool that makes it easy to perform routine screenings of MRSA.

## PRINCIPLE OF THE TEST

The Q-MRSA Detection Kit is a real-time PCR based assay for the detection of MRSA. Ready to use qPCR primer/probe mixes are included in the kit.

The Q-MRSA Detection Kit was developed based upon the platform of the Yin-Yang Probe, a novel technology for nucleic acid detection and related applications (Patent No. US7799522). The probe itself is non-fluorescent due to the close proximity of the fluorophore and quencher. When the Yin-Yang probe is in presence of its target DNA, the target strand displaces the negative strand along with its quencher, and the fluorophore becomes fluorescent.



In the process of qPCR, the positive probes bind to the PCR products during the annealing step, and release fluorescence signals. Signals are collected in the annealing step.

qPCR is performed in a real-time qPCR instrument with optical filters for the detection of FAM, HEX, ROX and Cy5. qPCR amplification plots and  $C_t$  (threshold cycle) values are used for identification of MRSA positive/negative samples.

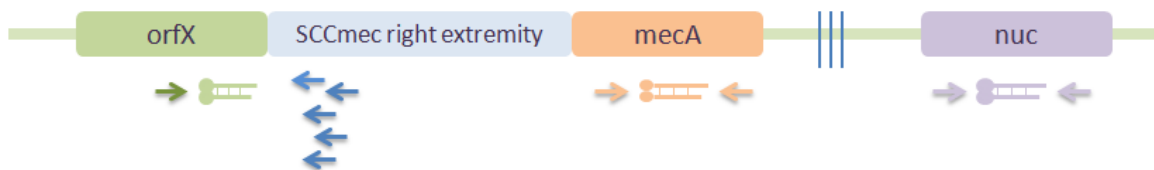


Figure 1: Schematic of the primers and probes designed in the MRSA screening kit. nuc, mecA and the orfX junctions regions are detected simultaneously. Primers are depicted in horizontal arrows.

## KIT COMPONENTS AND STORAGE

The kit must be stored at -20°C away from light. Avoid frequent/unnecessary freeze-thaw cycles of the kit.

		10-test kits	20-test kits
PCR REAGENTS	4X Primer/Probe Mix	70 µL	140 µL
	2X QD Supermix	140 µL	280 µL
	Internal Control	14 µL	28 µL
	Positive Control	10 µL	20 µL

## REQUIRED EQUIPMENT

- Table centrifuge
- Thermal heating block
- qPCR instrument with filters for FAM (Abs 495 nm, Em 520 nm), HEX (Abs 535nm, Em 556 nm), ROX (Abs 580 nm, Em 615 nm) and Cy5 (Abs 650 nm, Em 670 nm).
- Pipettes and sterile filter tips.

## PROCEDURE

### *Real-time qPCR*

Each 20 µL reaction contains 10 µL **2X QD Supermix**, 5 µL **4X Primer/Probe mix** and 1µL **internal control\*** and 4 µL DNA sample.

Note: It is important to add in the internal control to exclude PCR inhibition and to monitor reagent integrity.

- Make the master mix: If the number of sample DNA that need to be detected is number n, the volume of master mix needs to be at a minimum  $(n+3)* 10 \mu\text{L}$  QD supermix +  $(n+3)* 5 \mu\text{L}$  Primer/Probe mix +  $(n+3)* 1 \mu\text{L}$  Internal Control. (The extra 3 are for one positive control, one negative control, and one spare tube in case of pipetting error.)
- For every sample, pipette 16 µL master mixes to each reaction.
- Add 4 µL DNA sample to each reaction.

- Run the qPCR reaction with the following program, collect data at the 60°C annealing step:

	Thermal cycle	Cycle #
Preheating	95°C 3 min	1
Touchdown	95°C 20 sec	5
	65°C 30sec (decrease 1°C/step)	
	72°C 30 sec	
PCR Cycle	95°C 20 sec	35
	60°C 30 sec (4 channel detection: FAM, HEX, ROX and Cy5)	
	72°C 30 sec	

### Analysis and interpretation

All reactions should give ROX signals for the Internal Control, and the  $C_t$  values should be below 28. FAM, HEX and Cy5 signals should be detected for the Positive Control, and the  $C_t$  values should be below 28.

$C_t$  values for positive sample signals from FAM, HEX and CY5 channels should be below 32.  $C_t$  values above 32 might be non-specific amplification. Repeat the test with DNA of higher concentration if possible. Other diagnostic techniques are highly recommended to confirm the results from positive tests with  $C_t$  values above 32.

Channel	Genes Detected
FAM	SCCmec:orfX junction
HEX	mecA
CY5	nuc
ROX	Internal Control

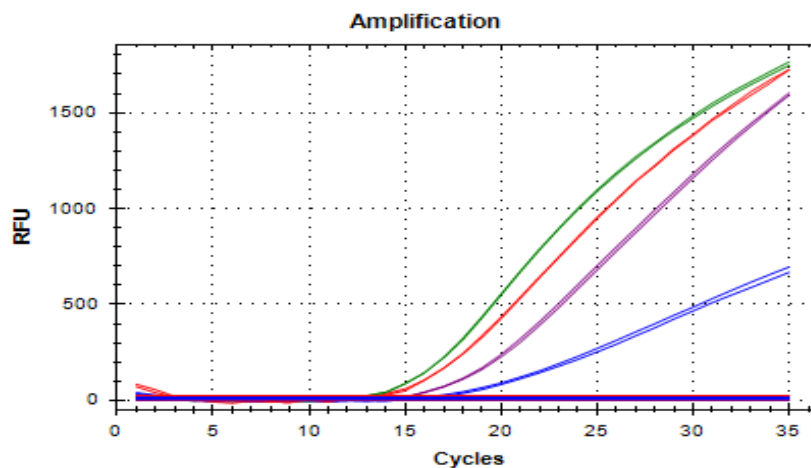


Figure 2: Example of the amplification curve of MRSA positive sample. Green: orfX junction region; Purple: nuc; Blue: mecA; Red: Internal Control.

## APPENDIX

### ***Program set-up for commonly used real-time qPCR instruments:***

ABI 7500, Agilent Mxpro 3005, Bio-Rad CFX96 and Qiagen Rotor-Gene.

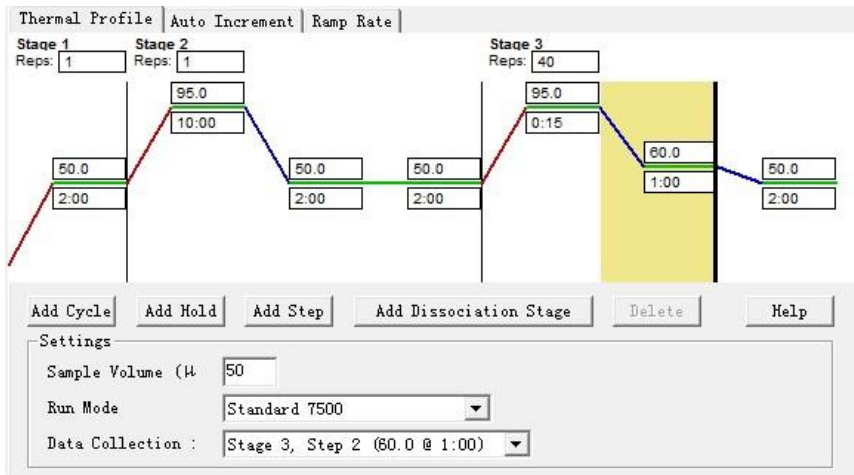
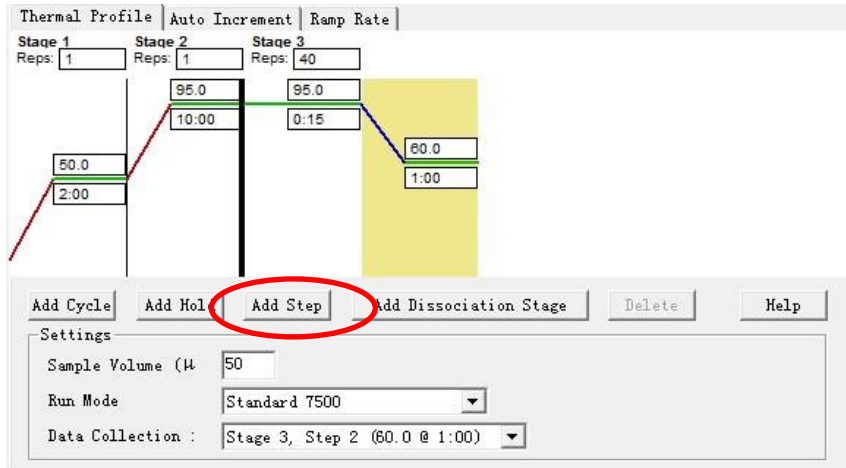
#### *qPCR program set-up for ABI 7500*

1. Log in to 7500 System SDS Soft , choose the right channel, click on INSTRUMENT, set up the program in the blank area as shown below:

The screenshot displays the 7500 System SDS Software interface. The 'Instrument' tab is selected and circled in red. The 'Instrument Control' section includes buttons for Start, Stop, Disconnect, and Extend, along with fields for Estimated Time Remaining and Status. The 'Thermal Cycler Protocol' section shows a graph with three stages: Stage 1 (50.0, 2:00), Stage 2 (95.0, 10:00), and Stage 3 (95.0, 0:15). A yellow shaded area highlights a step at 60.0 for 1:00. The 'Settings' section includes fields for Sample Volume (50), Run Mode (Standard 7500), and Data Collection (Stage 3, Step 2 (60.0 @ 1:00)).

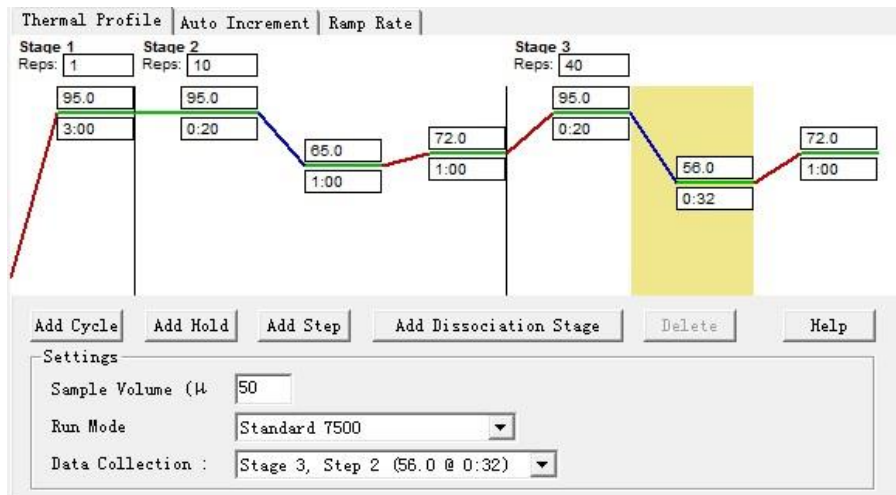
Stage	Temp (°C)	Time	Reps
Stage 1	50.0	2:00	1
Stage 2	95.0	10:00	1
Stage 3	95.0	0:15	40
Stage 3, Step 2	60.0	1:00	-

- Click on Stage 2, a black line will show up on the right side, then double click on Add Step for additional two steps. Also add one more step for Step 3.



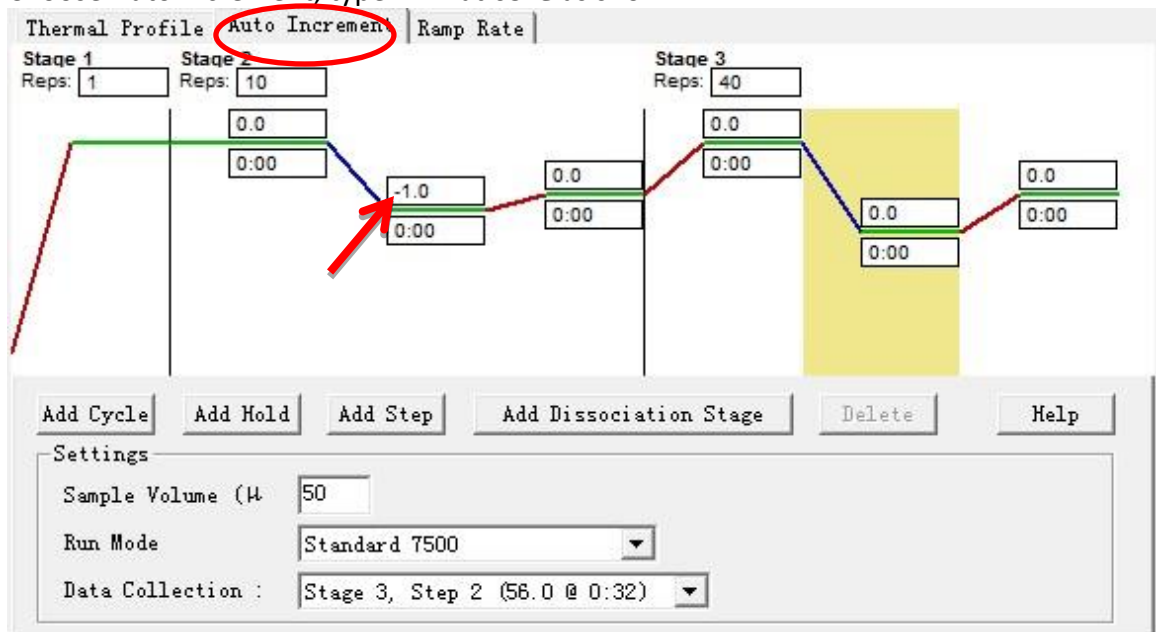
- Program modification

	Thermal cycle	Reps
Stage 1	95°C 3 min	1
Stage 2	95°C 20 sec	5
	65°C 30 sec	
	72°C 30 sec	
Stage 3	95°C 20 sec	35
	60°C 30 sec	
	72°C 30 sec	



4. Set up Touchdown

Choose Auto Increment, type in -1 at 65°C as shown

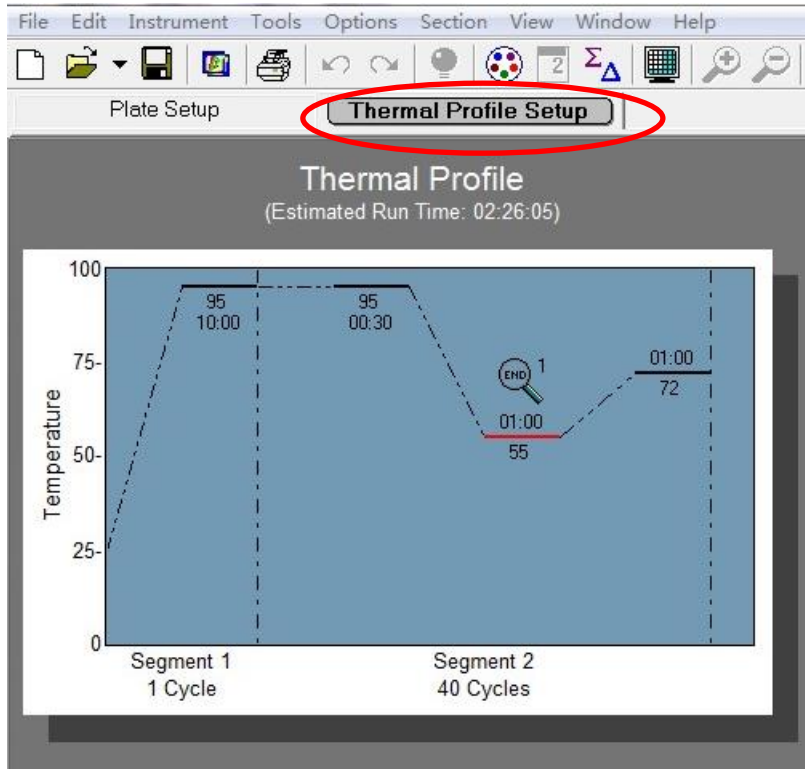


5. Set Sample Volume as 20, make sure the Data Collection is at Stage 3, Step 2.

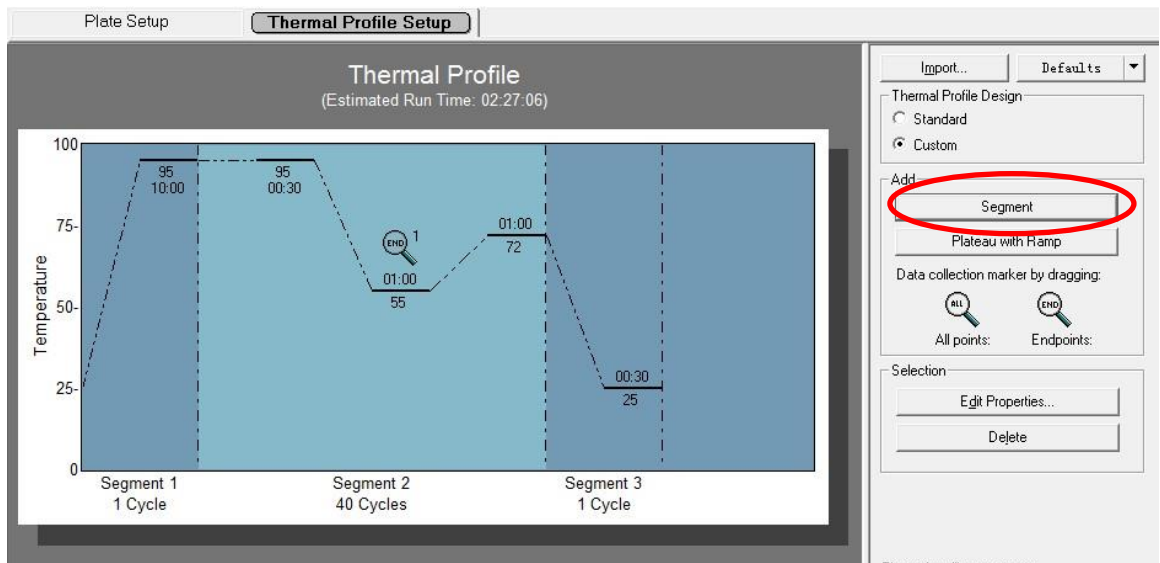
6. Save and Run

*qPCR program set-up for Mxpro 3005*

1. Choose Thermal Profile Setup

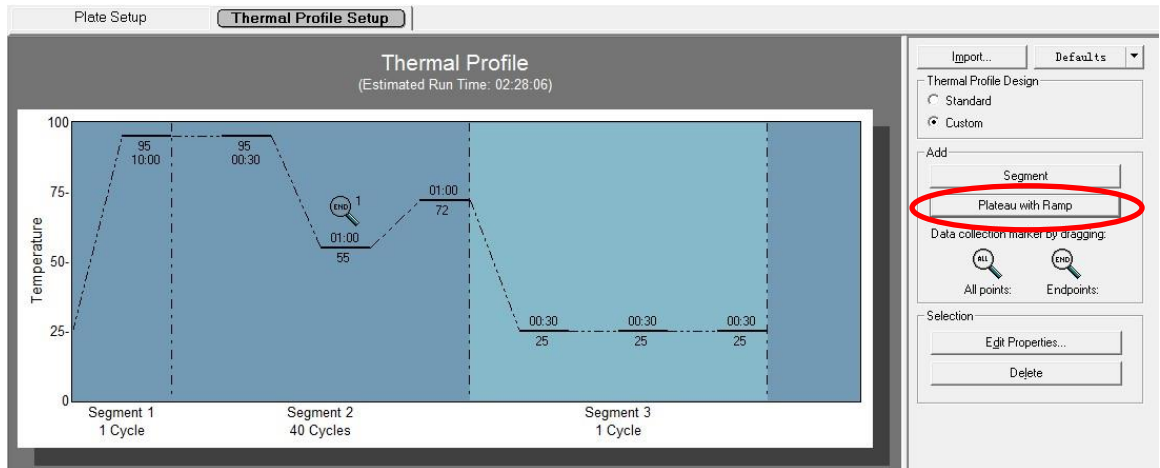


2. Choose Segment 2, click on Add Segment.



3. Choose Segment 3, double click on Add plateau with Ramp to add two steps.

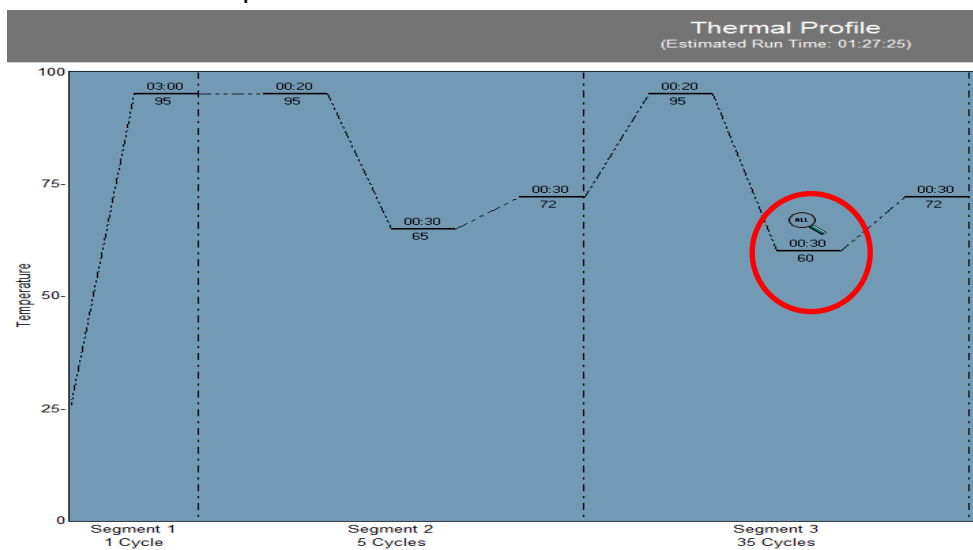




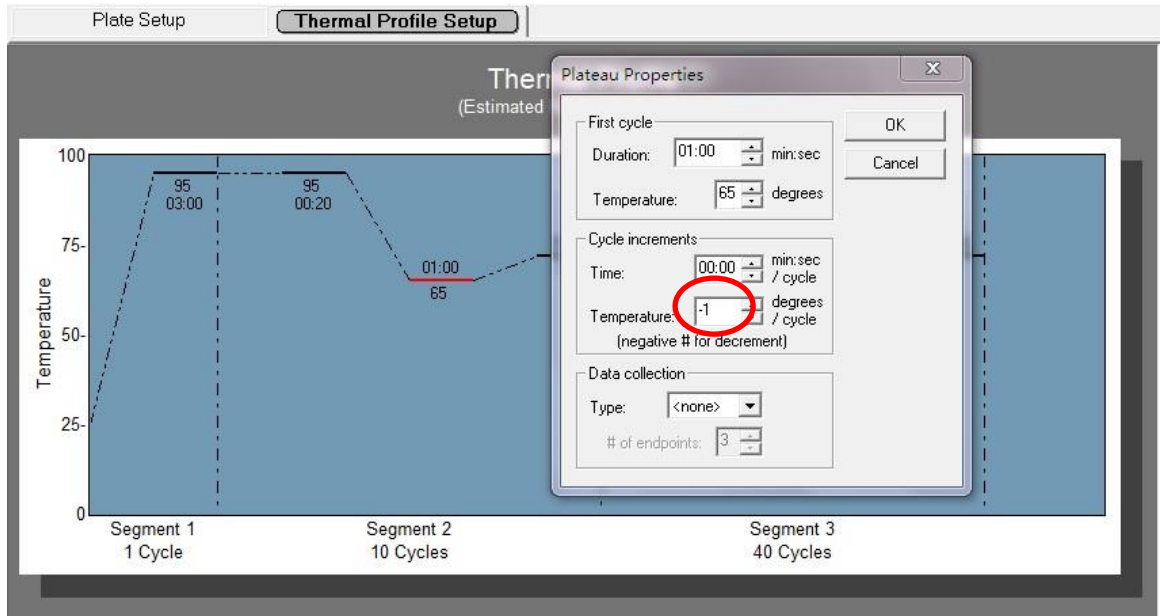
4. Program modification

	Thermal cycle	Reps
Stage 1	95°C 3 min	1
Stage 2	95°C 20 sec	5
	65°C 30 sec	
	72°C 30 sec	
Stage 3	95°C 20 sec	35
	60°C 30 sec	
	72°C 30 sec	

5. Move the data acquisition lens to 60°C 30 sec.



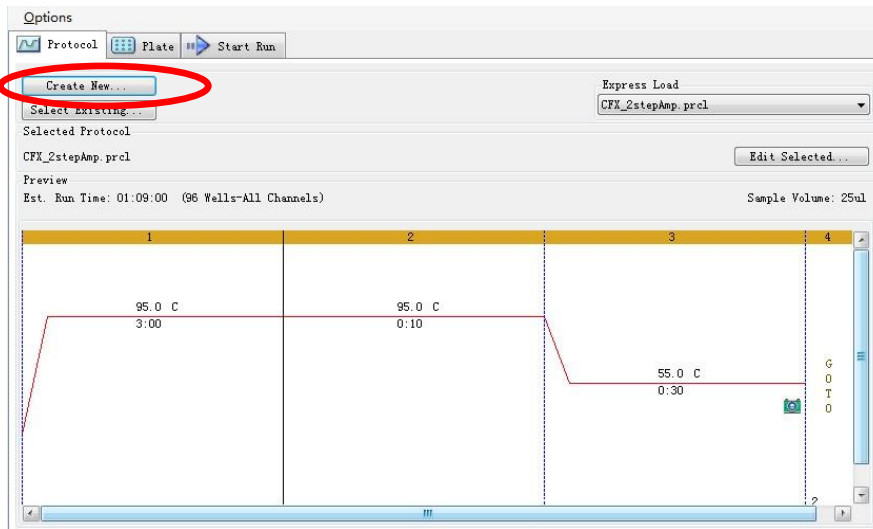
6. Add Touchdown program. Choose 65°C 30 sec and double click, within the pop-up window, type in -1 at Temperature inside Cycle increments, click on OK.



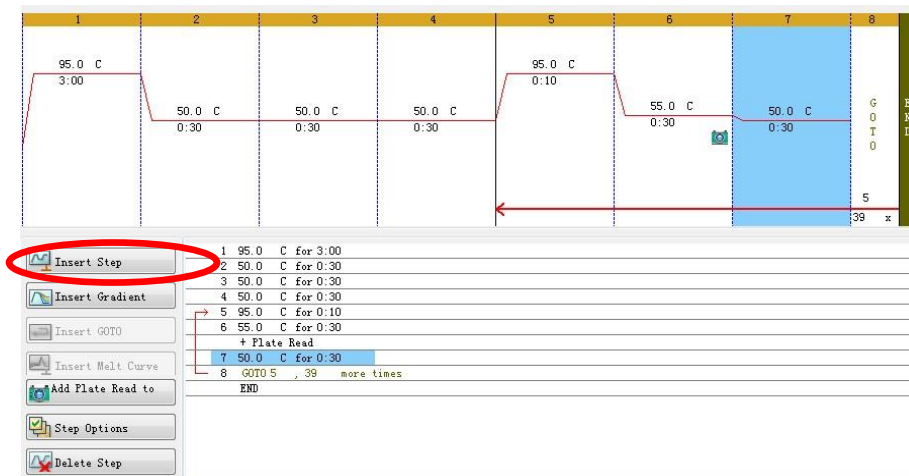
7. Save and Run.

## qPCR program set-up for Bio-Rad CFX96

### 1. Click on Create New



### 2. Choose 1, click on Insert Step three times to add three steps; choose 6, add one step

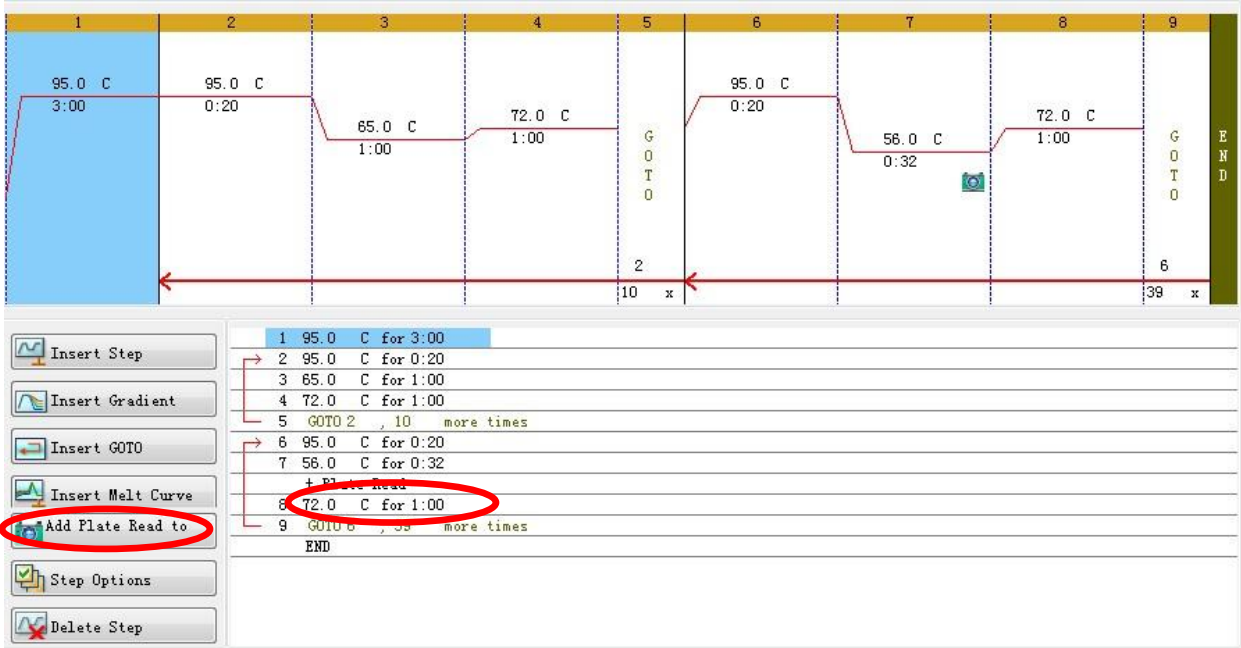


### 3. Change temperature and time:

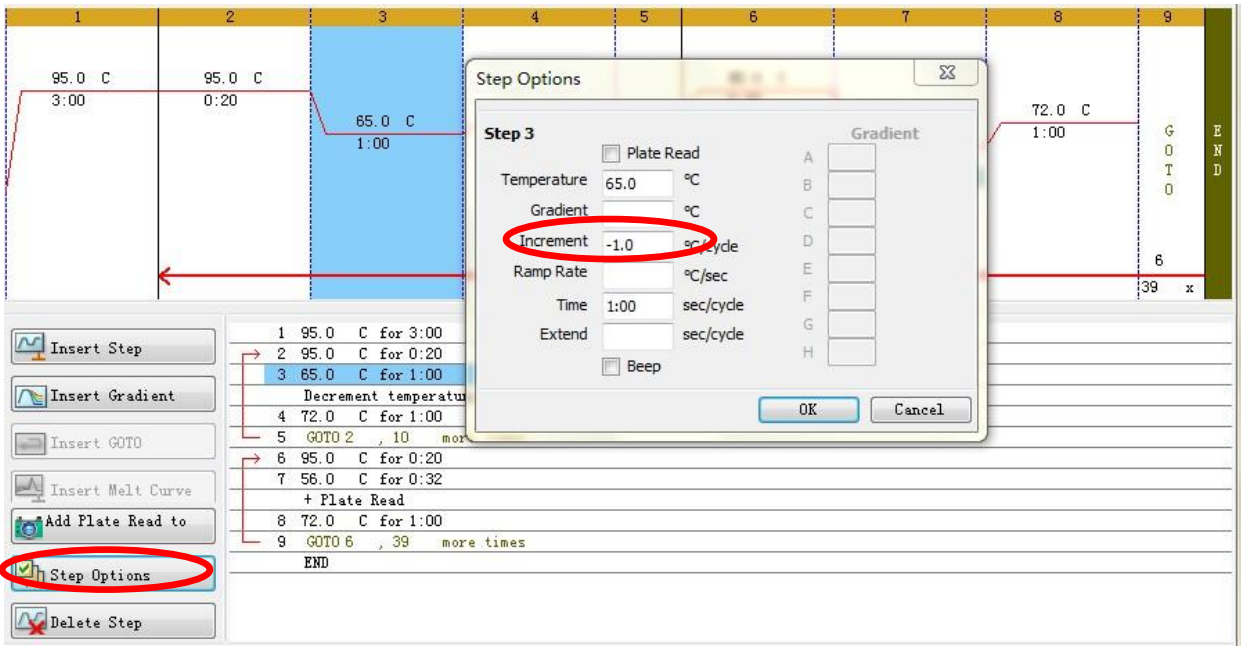
95°C 3 min, 4 more times (95 °C 20s,65 °C 30 sec,72 °C 30 sec)

34 more times (95 °C 20s,60°C 30s,72 °C 30 sec)

Choose 4, click on Insert GOTO, type in 2, 9 more times behind GOTO.



4. Add Touchdown: choose step 3, click on Step Options, type in -1 at Increment in the pop-out window, click on OK.



5. Make sure the data acquisition camera is placed at 60°C, 30 sec.

6. Save and run.

## qPCR program set-up for Rotor-Gene series instrument

1. Log into Rotor-Gene Series Software, choose “Advanced”, select “Empty Run” and then click on “NEW” (Figure 1).

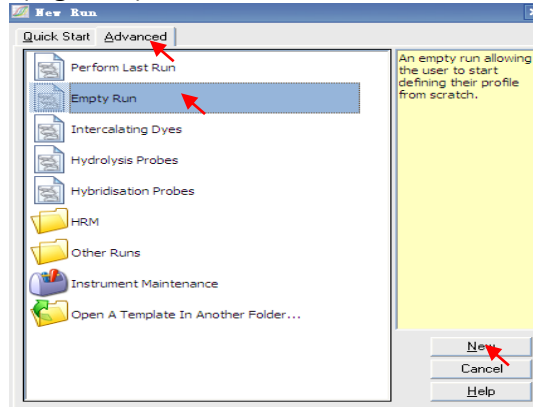


Figure 1

2. Choose “Rotor Type” according to the type of the instrument. Select the “Locking Ring Attached” to make sure the ring has been put on the rotor, then click on “Next” (Figure 2).

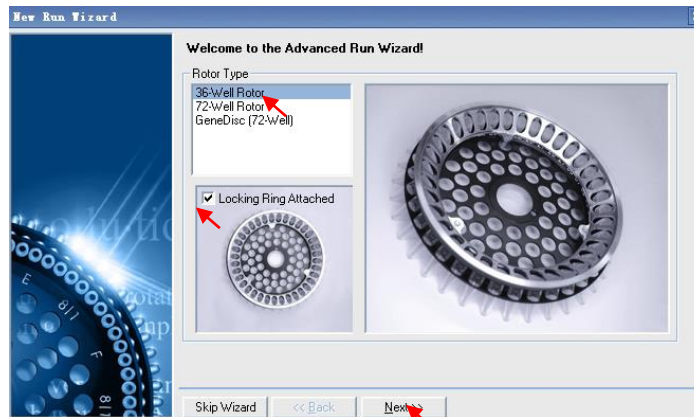


Figure 2

3. Type in the name of the “Operator”, fill in the “Reaction Volume” as 25  $\mu$ L, and then click on “Next” (Figure 3).

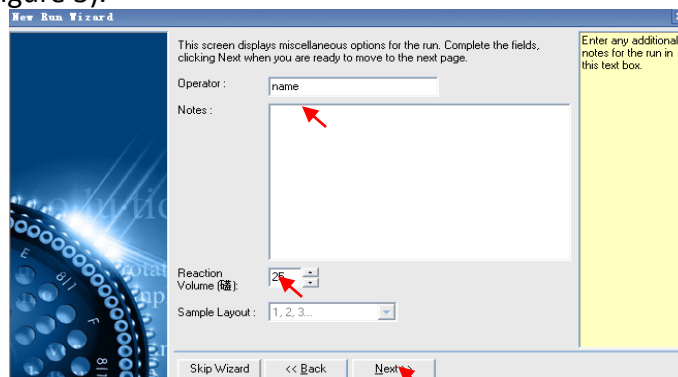


Figure 3

4. Click on “Edit Gain” to modify the gain values of the Green, Yellow, Orange and Red channels (Figure 4). Gain value 5 is recommended for each channel. Click on “OK” to complete the gain set-up (Figure 5).

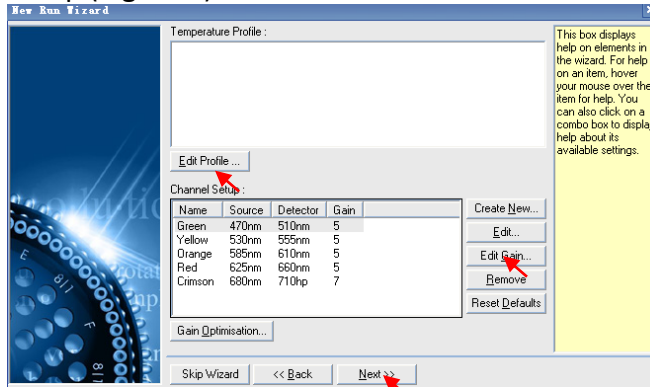


Figure 4

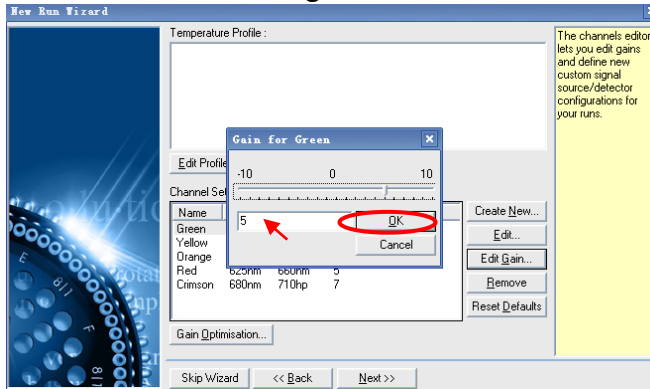


Figure 5

5. Choose “Edit Profile” as shown in Figure 4. Click on “Insert after” to insert the three thermal stages: “Hold”, “Cycling” and “Cycling2”.

a. For the hold stage, select “Hold”, click on “Hold Temperature” to set the temperature as 95 degrees, click on “Hold Time” to set the time as 3 mins (Figure 6).

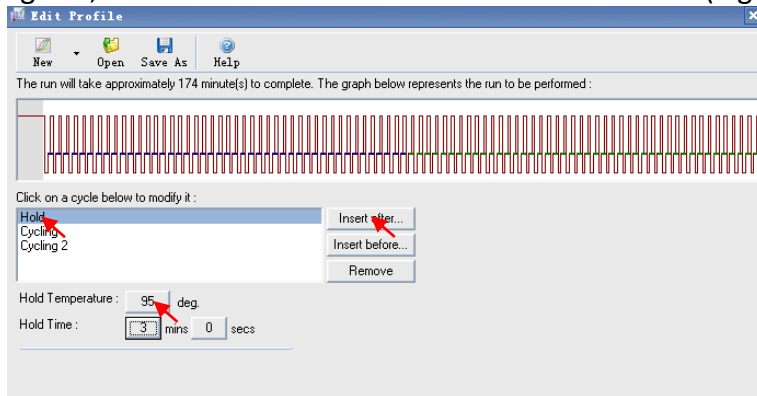


Figure 6

b. For the cycling stage, first choose “Cycling”, click on “this cycle repeats times” and set as 10 times, then click on “OK”(Figure 7).

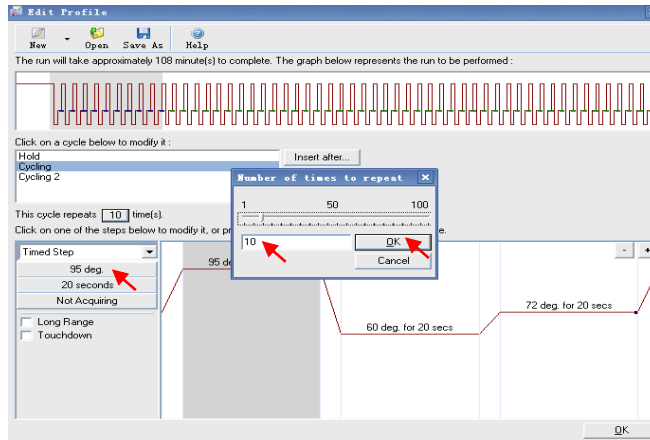


Figure 7

c. Choose the first step of cycling, click on buttons on the left and set the temperature as 95 degrees, time for 20 seconds, choose “not acquiring” for this step, and then click on “OK” (Figure 8).

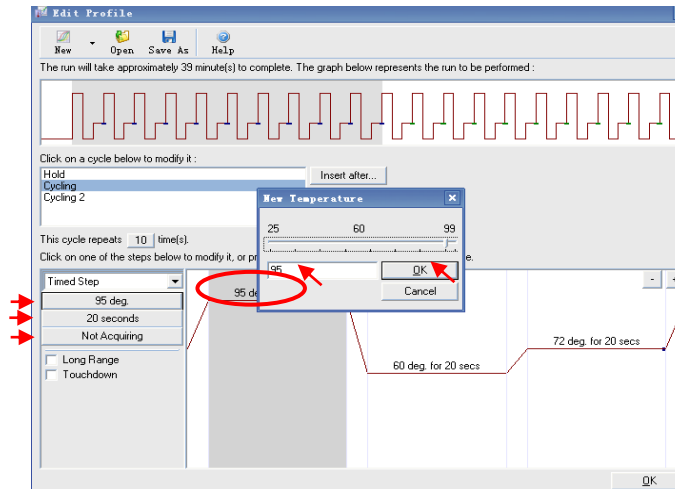


Figure 8

d. Choose the second step of “cycling”. Set the temperature as 65 degrees, set the time as 30 seconds, choose “not acquiring” and click on the “touchdown”. In the “Cycles to touch down”, set the “Decreases temperature” by 1 degree for each cycle and 5 cycles, click on “OK” to complete the set-up of touchdown (Figure 9).

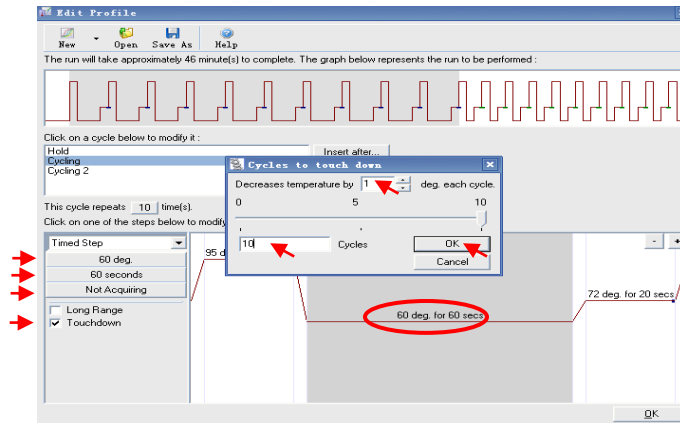


Figure 9

e. Choose the third step of cycling, set the temperature as 72 degrees, set the time as 30 seconds and choose “not acquiring”. Click on “OK” to complete the set up of the cycling stage (Figure 10).

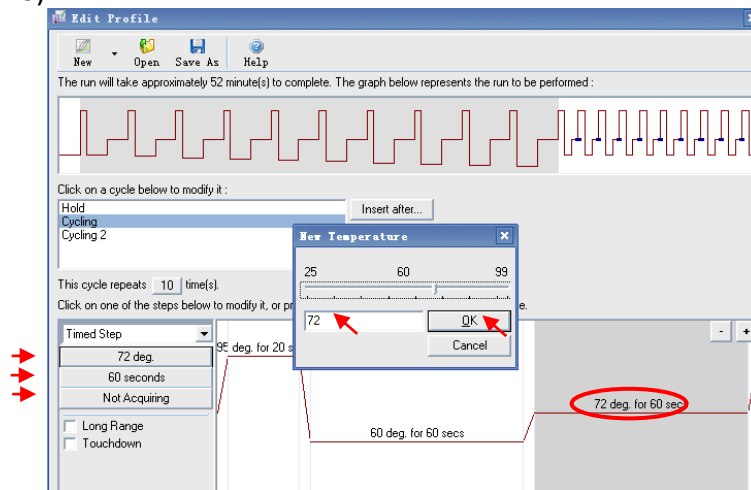


Figure 10

6. For the stage of “Cycling2”, choose “Cycling2”, set the temperature and time as below for each step: 95 degrees for 20 secs; 60 degrees for 30 secs and 72 degrees for 30 secs. Make sure “not acquiring” is selected for the first (95 deg.) and the third step (72 deg.). Data is acquired at the second step (60 deg.)

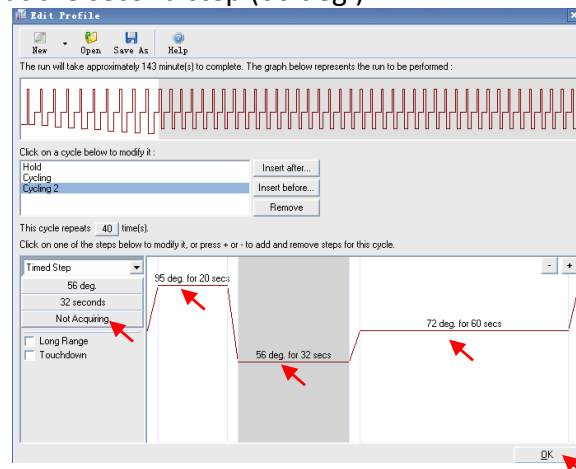


Figure 11



Click on the “Not Acquiring” button. In the “Acquisition”, use the > to shift the Green, Orange, Red and Yellow channels in the “Available Channels” into the “Acquiring Channels”, click on “OK” to complete the set-up of this part (Figure 12). Then click on “OK” as shown in Figure 9.

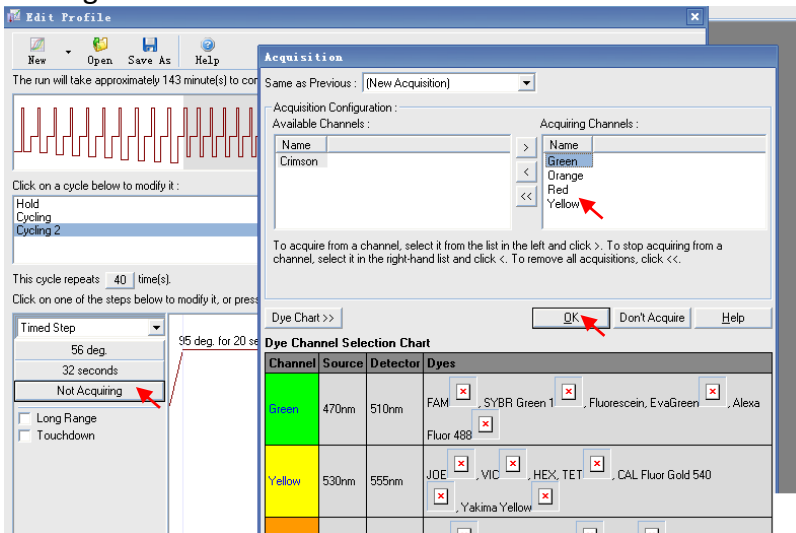


Figure 12

7. Click on “Next” (Figure 13) and “Start Run” (Figure 14) to save the file into the computer and finish the set-up.

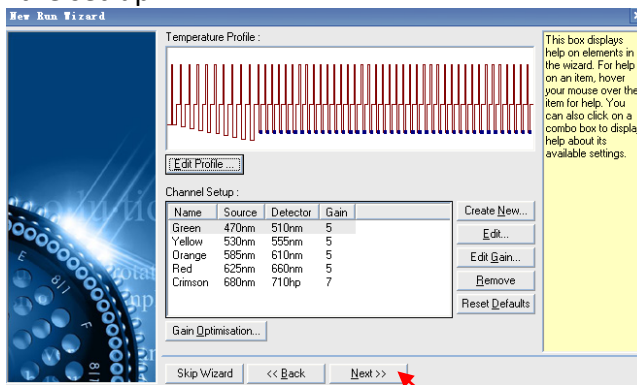


Figure 13

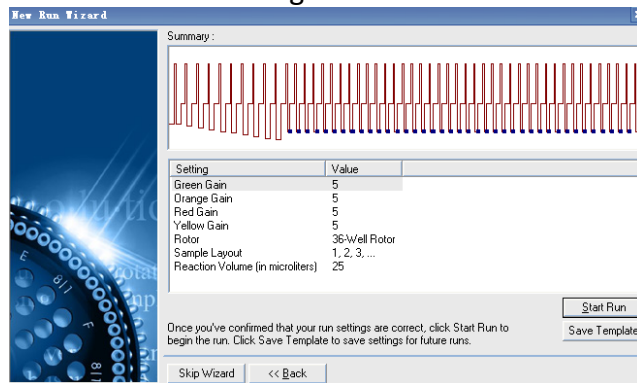


Figure 14