

REAL TIME qPCR SERVICE

The Molecular Biology Service is equipped with a high throughput instrument for the Real Time qPCR. Such instrumentation uses 384 well plates which not only allows to analyze an high number of samples in the same run but also to reduce the reaction volumes resulting in a reduced cost per reaction. To use such technology is necessary to book on the SBM web pages (www.sbmweb.it). In every working day there are four available runs qPCR according to the following calendar:

1. Turno **1** starting run at 9:30
2. Turno **2** starting run at 10:45
3. Turno **3** starting run at 12:00
4. Turno **4** starting run at 13:15
5. Turno **4** starting run at 13:15
6. Turno **4** starting run at 14:30
7. Turno **4** starting run at 15:45
8. Turno **4** starting run at 17:00

We thank you to avoid consecutive bookings if possible.

The instrument can directly be used by the user using the following "**quick setup and run guidelines**". We recommends for the first time to ask instructions to the SBM staff. Reaction volumes and thermal profile suggested and validated by the SBM with the available chemistry are reported.

Quick setup and run guidelines

Setup plates according to the "**standard SBM protocol**"

<i>Add Order</i>	<i>Sample</i>	
<u>1</u> °	SYBR Master Mix	5,0 ul
<u>2</u> °	Oligo F + Oligo R [0,7 pmol/ul] each	4,0 ul
<u>3</u> °	cDNA	1,0 ul
	V tot.	10,0 ul

Reaction plates will be analyzed according to the **standard** or **Fast** SBM thermal profile

Thermal profiles

Standard SYBR (duration ≈ 1 h 50 min)			Fast SYBR (duration ≈ 50 min)		
Step 1	95°-10 min		Step 1	95°-10 min	
Step 2	95°-15 sec		Step 2	95°-1 sec	
Step 4	60 °- 1 min	39 times	Step 4	60 °-20 sec	
Go to	2		Go to	2	39 times
Step 6	Melting	60°-95°	Step 6	Melting	

Setup and run:

1. Power on the instrument with the button on the back left side
2. Press the red button in the lower right corner of the touch screen. From the right side of the instrument the plate loader tray will appear
3. Place the plate in the right orientation (A1 in the upper left corner) **properly on the tray.**
4. Press again on the touch screen the red lower right corner button to load the plate
5. On the main screen in the "my shortcuts" section press, **according to the SYBR you are using:**
 - "SBM_SYBR_delta_delta_ct_standard_10ul_no_rox" **for standard SYBR without ROX**
 - "SBM_SYBR_delta_delta_ct_FAST_10ul_rox" **for Fast SYBR with ROX**
 - "SBM_SYBR_delta_delta_ct_FAST_10ul_no_rox" **for standard SYBR without ROX**
6. in the "notes" section insert your name using the touch keyboard that will appear
7. press "start run now"

Downloading the result file:

1. Be sure that the run is completed (check run time)
2. In the main window of the touch screen press "collect results"
3. Insert the USB memory stick in the left side of the touch screen
4. select the appropriate run file
5. press "copy to USB"