

## SYBR Green RT-PCR Protocol

The sensitivity was 100-10 picograms with this protocol and gave nice curves.

### **Materials**

ROX Passive Reference Dye 25  $\mu$ M, 50x (Invitrogen, Cat. No. 12223-012)

SYBR Green I, "10,000x" concentrate (Molecular Probes, Cat. No. S7563)

Titanium Taq polymerase 5 U/ $\mu$ L (Clontech, Cat. No. 639242)

### **Solutions**

SYBR Green 1:100 dilution (50  $\mu$ L SYBR Green concentrate, 5 mL DMSO, store aliquots at -20°C in dark tubes in a dessicator)

SYBR Green 1:2000 solution (0.5 mL of SYBR Green 1:100 dil., 1 mL glycerol, 10  $\mu$ L Tween, 8.5 mL H<sub>2</sub>O, store at -20°C in dark tubes)

<i>KG-1 (10x)</i>			<i>KG-2 (10x)</i>	
<u>Amount</u>	<u>[final]</u>		<u>Amount</u>	<u>[final]</u>
8.3 mL 1M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	166mM		50 $\mu$ L of 100mM dNTP Stocks (x4)	(@10 mM)
33.5 mL Tris pH 8.8	670 mM		50 $\mu$ L DMSO	10%
174 $\mu$ L B-ME	50 mM		50 $\mu$ L BSA (8 mg/mL stock)	0.8 mg/mL
3.35 mL 1M MgCl <sub>2</sub>	67 mM		350 $\mu$ L H <sub>2</sub> O	
q.s. to 50 mL with H <sub>2</sub> O				

Keep tubes of master mix wrapped in foil to protect it from the light.

### **rtPCR for a 40ul reaction volume**

-> Master mix (N+1); N=number of reactions doing

1 ul primer 1 (1.75uM)

1 ul primer 2 (1.75uM)

4ul KG1

4ul KG2

10ul Sybr green 5x

0.8ul Rox

1ul Polymerase Titanium

17.2ul H<sub>2</sub>O

-> 39ul/well

1ul DNA or H<sub>2</sub>O

-->add 50ul mineral oil on the top with a repeater pipet (to minimize pipetting differences).

--> spin down in centrifuge 200g, for 1 min

## **PCR Cycling Conditions**

stage 1: 94C - 2 min

40 cycles stage 2: 95C - 15 sec  
60C - 30 sec  
72C - 30 sec  
81C - 5 sec

stage 3: 60C - 15 sec

ramping time 19' 59"

stage 4: 95C - 15 sec

data points:

stage 2 at 72C and 81C

stage 3 at ramping

-> file: "New plate"

- 1) Preferences: single reporter, Standard plate, real time, Online Documentation, PCR volume:40ulSample setup
- 2) Unknown is "SYBR" and unselect "Quencher"Select wells that have the samples. Only a large square can be selected.
- 3) Analysis -> Options -> uncheck "auto compute PCR stage"  
-> check "show threshold Dialog After Analyze"
- 4) User Defined -> PCR Stage Collection time-> stage 2; Extension step 3
- 5) Instrument -> Diagnostics -> Advanced Options  
-> Viewer -> check both "raw" and "multicomponent"
  1. -> Misc -> set to 25ms
    1. -> reference Check "Rox"
  - 1) Program will say restart to use settings => DISREGARD
  - 2) place plate in with foam padding (gray foam facing top of plate) and secure in machine.

-> can save these settings by "save as" and give new template name

## **Analyzing Data**

-> Analyze

-> look at the data at "rxn vs.baseline"

-> set the baseline by clicking on the deltaRxn axis. A window will appear allowing one to change from linear to logarithmic scale. Change to liner scale. Note at which cycle number the signal starts to increase. This cycle will be the end of the baseline value.

-> change the scale back to logarithmic scale

-> change the baseline end value to the one determined from the linear scale

It is easier to analyze one set of data at a time.

-> For one set of data, set the threshold by clicking on the line and moving it upwards or downwards to be in the linear range. Check that the CT values are not at background (40).

### **Exporting Data from MAC to computer**

-> after the analysis has been optimised, the threshold settings and CT values for them can be saved and sent over. If one wants to save data sets with different settings, you can exports multiple different sets of analysis.

-> file -> Export “results”

-> give file a name

-> file will be saved on desktop

-> Software folder on desktop -> “MacRAR 2.0.1 fat”

-> drag in exported results that was saved on desktop to the MacRAR window

-> “save as” -> give file a name