R3Charge Real-time Master Mix Protocol

For best results, keep all reagents on ice (or at 4˚C) at all times during set up.

Real-time qPCR amplification and detection

1. Gently thaw template DNA, primers and R3Charge (2X) on ice. Vortex and spin down reagents.

2. If using cDNA templates directly from an RT reaction, dilute the template 1:10 in nuclease free water. Load template DNA into plate.

3. Assemble qPCR reaction according to Table 1. Dispense appropriate volume of qPCR master mix per well.

Table 1 – Real-time qPCR setup, individual assays (96 well-plate)

Reagent	Volume
qPCR Master Mix (2x)	10 µl
qPCR Primers and Probes	(suggested final primer concentration 0.2 µM)
Nuclease-free water	to 20 µl
Template DNA	X µl
Total volume	20 µl

4. Centrifuge the PCR plate briefly (30 s at 200 g).

5. Perform Real-time PCR amplification with the following cycling parameters.

Table 2 – qPCR fast thermal cycling protocol

Cycles	Temperature	Time	Notes
1x	95˚C	5 min	Polymerase activation
40x	95˚C / 98 ˚C	10 s / 1s	Denaturation
	60˚C / 60˚C	30 s / 5s	Annealing/extension (acquire florescence reading at end of step) For amplicons >500bp, increase the annealing/extension time up to 1 min.

6. Data analysis

Ordering information

Real-time PCR Instrument	ROX requirement	Cat. No.	
		2X Green MM	2X Probe MM
Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® MIC qPCR Bio-Rad® iQ™5, CFX96, CFX384, Opticon Aperbio Pangaea	Not required	DS1272113 (600) DS1272114 (1,200) DS1272115 (2,400)	DS1272153 (600) DS1272154 (1,200) DS1272155 (2,400)
Applied Biosystems® 7500, QuantStudio™ 3, 5 and 7 ViiA7™ Agilent Mx™	Low ROX		DS1272133 (600) DS1272134 (1,200) DS1272135 (2,400)
Applied Biosystems® 7000, 7300 7700, 7900HT, StepOne™, StepOnePlus™	High ROX	DS1272123 (600) DS1272124 (1,200) DS1272125 (2,400)	DS1272143 (600) DS1272144 (1,200) DS1272145 (2,400)