

Marcu Lab Taqman Real time PCR protocol 03/05

This protocol uses the Qiagen QuantiTect Probe PCR Kit (Cat # 204343) and sequence-specific probes, labelled with a Fam or Vic dye, to identify the copy number of genes of interest, normalized using the copy number of GADPH (a housekeeping gene). The copy number is derived by the software of the machine, that builds a standard curve using a standard genomic DNA (gDNA) with a known number of copies of the gene of interest as a reference. The gDNA is used either undiluted, or diluted 1:100 and 1:10000 to build the standard curve.

Basic Protocol:

- 1) Retro-transcribe 2 ug of RNA (possibly purified thru Qiagen Rneasy Mini kit, cat # 74104) and dilute the cDNA 1:12.5 before using it for the Taqman reaction.
- 2) Mix the standard master mix and dispense it into standard wells
- 3) Mix experimental master mix and dispense it into experimental wells

Master Mix For 1 Reaction

2X PCR MasterMix	12.5 ul
Forward Primer (5uM)	0.5 ul
Reverse Primer (5uM)	0.5 ul
Probe (5 uM)	0.5 ul
cDNA (1:12.5) or gDNA	1.6 ul 2 ug
dH ₂ O	To 25 ul

- 4) Dispense standard DNA and experimental DNA into the corresponding wells
- 5) Spin plate down for 1' at 2000 rpm and place it in the MX3000P Thermal Cycler
- 6) Set up the plate
- 7) Set up the Thermal Program:
 - Cycle 1 (1X) 50°C for 02:00
 - Cycle 2 (1X) 95°C for 10:00
 - Cycle 3 (50X)
 - Step 1: 95°C for 00:30
 - Step 2: 60°C for 01:00

Data collection at the end of each cycle

Analysis of the data

Calculate the median value of the copy numbers of your experimental data. Divide it by the GADPH value of each experimental sample. This is the Correction Factor for each experimental data. Multiply it by the corresponding experimental data. This is the corrected data.