

qRT-PCR setup

Reagents Needed:

- RNA (50-1000 ng/ μ l)
- cDNA synthesis kit (Invitrogen superscript II or III)
- TaqMan assay probe sets
- Applied Biosystems qPCR master mix

Protocol

1. Purify RNA using the standard 'Worm RNA Isolation' protocol
2. Check purity and quantity of RNA on the spectrophotometer (pure RNA has a 260/280 ratio of ~2.0; dilute 1 μ l into 100 μ l of water (1:100 dilution).
3. Reverse transcribe 100-1000 ng RNA into cDNA using the superscript kit, following the directions appropriate to that kit. Measure the volume of the cDNA sample (ideally use 1 μ g of RNA as template. If RNA concentrations are too low, determine the amount of RNA added using the lowest sample concentration and add this amount of RNA for all other samples.)
4. Check cDNA yield on spectrophotometer (1:100 dilution) - should be ~1 μ g/ μ l.
5. Set up qPCR reaction as follows:
 - First, dilute cDNA solution 2.5ng/ μ l (if you used 1 μ g of RNA, the concentration of the superscript reaction is 47.6ng/ μ l (1000ng / 21 μ l reaction volume. Dilute reaction 1:19 (10 μ l cDNA / 180 μ l water) to reach 2.5ng/ μ l
 - Each reaction contains:

Component	Volume per reaction
20x TaqMan assay probe set	1.0 μ l
cDNA (2.5ng/ μ l)	9.0 μ l
2x universal master mix	10.0 μ l
Total reaction volume	20μl

- Make master mixes for each probe set to be tested (master mix contains the TM assay and master mix)
 - Layout your reaction template using the attached worksheet prior to adding any reagents. Each sample should be run in at least triplicate and you must run an endogenous control for each cDNA.
 - Add probe set master mix (11 μ l / well) + cDNA (9 μ l/well) into a 96 well qPCR plate
 - Seal the plate with the optical sealing film and sealing tool.
 - Run in the CFAR APB7500 machine in Johnson Pavillion, 5th floor
6. Analyze data on lab computer using AP software package.

qPCR setup

Gene(s) of interest probe: _____

Date: _____ Endogenous ctrl probe: _____

Calibrator sample: _____

Experiment: _____ Experimental sample(s): _____

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

NOTES: