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Quantitative PCR, 384 well format

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We use this protocol and it's working

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Abstract

Quantitative PCR, 384 well format

Materials

Materials:

- PCR 8-tube strips or 96 well plates depending on sample# and gene# (*General stores 4868*)
- PCR strip tube lids (*General stores 1400-0800*)
- ABI Sybr Green (*ABI 4301955*)
- QPCR plate (*ABI 4309849*)
- Adhesive plate film (ABI 4311971)
- Brown plastic adhesive helper
- Filtered 200 µl pipette tips (2 boxes for one complete plate)
- Electronic Multichannel Pipette (referred to as Hilda from here on out)
- Primers – final concentration of 1.25 µM each
- Autoclaved MilliQ H_2O

Set-up:

1 Sample **cDNA**: thaw your cDNA on ice (*vortex and quick spin before plating*)

1.1 Primer stock (+/-): Add 12.5 µl of (+) and 12.5 µl (-) to 1 mL MilliQ H_2O

1.2 PCR Master Mix (10ul rxn; Triplicate)

	A	B
	MilliQ H2O	10.20ul
	SYBR Green	20ul
	primers (2.5 µM each set)	4.80ul
	Total master mix	35.00ul

Equipment: ABI 7900 Prism

Procedure:

2 Place 8-tube PCR strips in PCR tube racks (each single tube runs 1 sample and 1 gene)

3 Add 5 µl cDNA to the bottom of each tube, use 20 uL pipetor (keep on ice)

4 Add 25 µl (duplicates) or 35 µl (triplicates) of Sybr Mastermix to the 8-well tube (keep on ice)

4.1 Use 200uL multi-channel pipette.

5 Mix using the multichannel and quick spin.

6 Dispense 10 µl of cDNA/Mastermix (20uL pipetor) into each well on 384 well plate according to plate layout made in advance (keep plate on ice)

7 Gently blot top of plate with kimwipe (to keep samples from transferring to other wells)

8 Place clear Adhesive plate cover over the plate.

8.1 use brown ‘helper’ to smooth out

8.2 pay attention to edges

8.3 work from center of the plate out

9 Spin plate for 5 min at 3500 rpm (4° C)

9.1 During spin: set up ABI SDS program (keep plate in centrifuge until ready to run)

10 Seal plate with sticky film. Vortex and spin down plate 3500rpm for 5 min at 4C

11 Open SDS 2.3 program

11.1 File -> new

11.2 One instrument tab: real time -> Connect to machine -> open/close door

11.3 Insert plate, aligning A1 to A1

11.4 Close door

11.5 On layout tab; highlight unused wells, click “omit wells”

11.6 Highlight used wells and click “add detector” for each specific gene

11.7 Set to 10uL Rxn VL

11.8 Check cycle times and temperatures

11.9 Add dissociation stage (SYBR primers only)

	A	B	C	D	E	F	G	H
	Temp C	50	95	95	60	95	60	95
	Time	2:00	10:00	0:15	1:00	0:15	0:15	0:55

Stage C&D 40 time

12 Run plate

13 Primer Validation Procedure: Set-up is same as above plus cDNA standard curve for each gene in an extra set of 8-tube PCR strips (see workflow file)