

qPCR Protocol

- 1) Dilute cDNA sample to 10^{-3} with nuclease free water
- 2) Set up qPCR plate on cold plate
- 3) Pipet reaction into each well in order of: Master Mix → probes → cDNA

qPCR Reaction Mix Components → Simplex PCR setup for 20 uL reaction

<u>Component</u>	<u>Volume (uL)/ Reaction</u>
TaqMan Gene Expression Master Mix	10
Assay (probe)	1
Diluted cDNA	9
20 uL	

- 4) Seal the plate and spin down approximately 3 min at 1000-2000 rpm to get rid of bubbles
- 5) Run on thermocycler TaqMan program

Note: When choosing probes use

1. NSR1 = cold shock +
2. APL3 = housekeeping
3. NUP145 = housekeeping
4. RPB4 = housekeeping
5. Choose 1 experimental probe