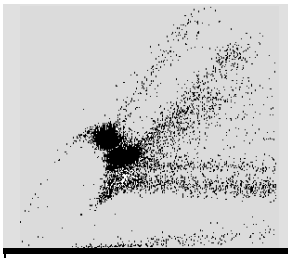

THE GOODELL LABORATORY

Author	Nathan Boles	Feb., 2009
Title	**100 Cell Equivalents Real-Time PCR**	
Introduction	This protocol describes 100 cell equivalents Real-time PCR.	
Materials	1. 10 mM dNTP 2. 500ug/mL Random primer mix 3. dH_2O 4. 5X 1st strand buffer 5. Rnase inhibitor 6. NP40 7. 2X Taqman Master Mix 8. 18s Taqman probe 9. Taqman probes for your gene of interest (GOI)	
Protocol		*Notes*
1.	Prepare the stock random primer mix: 1. 40 uL 10mM dNTP 2. 20 uL 500ug/mL Random primer mix 3. 20 uL dH_2O Dilute 1:24 to make stock random primer mix	
2.	Prepare lysis solution: 1. 167.2 uL dH_2O 2. 44 uL 5X 1st strand buffer 3. 4.4 uL of Rnase inhibitor 4. 1.1 uL NP40 5. 3.3 uL of stock random primer mix	*Makes enough solution for 4 wells of 50 uL*
3.	Pippet 50 uL of Lysis solution into each 4 wells of a 96 well plate	
4	Sort 1250 cells into each well, cover with optical cover sheet and bring back to lab	*Enough for 5 sets of 2 replicates. Or enough to compare expression of 2 genes between two groups*

5	After sort, pippette 1.5 uL of Superscript II into each well. Then do a quick spin of the plate to collect all liquid at the bottom of the well.	
6	Run plate on a PCR machine using a standard RT-PCR protocol.	
7	While RT-PCR is running prepare following master mix (does 20 wells) in 1.5mL tubes: 1. 110 uL 2X taqman master mix 2. 11 uL 18s taqman probe 3. 11 uL GOI probe 4. 70.4 uL dH_2O	*Makes enough 4 four wells which translates to 2 replicates of a single gene in 2 groups. If your situation doesn't fit scale it up.*
8	Split master mix in half (101.2 uL) to make the final mix using 1.5mL tubes	
9	After RT-PCR is finished, take 8.8 uL from your cDNA and add to the final mix. Mix by pippette, then spin down.	*8.8 uL is equivalent to 220 cells.*
10	Pippette 50 uL from final mix into each well.	
11	Run plate on ABI real-time system using standard real-time pcr protocol	