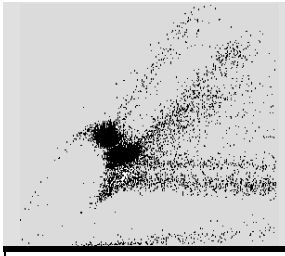

THE GOODELL LABORATORY

Author	Nathan Boles	Feb., 2009
Title	**Single Cell Real-Time PCR**	
Introduction	This protocol describes single cell 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. 608 uL dH_2O 2. 160 uL 5X 1st strand buffer 3. 16 uL of Rnase inhibitor 4. 4 uL NP40 5. 12 uL of stock random primer mix	
3.	Pippet 8 uL of Lysis solution into each well of a 96 well plate	
4	Sort a single cell into each well, cover with optical cover sheet and bring back to lab	…

5	After sort, take 10 uL of Superscript II and mix with 190 uL of dH_2O. Now pippette 2 uL of mix 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): 1. 275 uL 2X taqman master mix 2. 27.5 uL 18s taqman probe 3. 27.5 uL GOI probe	*20 individual cells per gene is reasonable, you can do more if you like just scale up the master mix.*
8.	After RT-PCR reaction is done add 15 uL of master mix to each well	
9.	Run real-time on ABI real-time system using standard real-time template	