Reverse Transcription protocol Eric Greer 9/2/09

Take 2 ug of total RNA sample in 9 λ total volume
Add 0.5 λ Dnase
Add 0.5 λ RNase OUT (I make a master mix of the two and add 1λ)
Put @ 37° for 15 minutes
Move to 75° for 15 minutes
Quick spin
Keep 9 λ for cDNA and remove 1 λ as control for RNA (will add 9λ dH_2O to equalize volume)
To 9 λ add 1λ dNTP and 1λ OligodT primers (again I add 2λ of a master mix)
Put at 65° for 5 minutes
Put on ice
Quick spin
Add 9λ of master mix

4λ 25mM $MgCl_2$
2λ 10X RT buffer
2λ 0.1M DTT
1λ RNase OUT
9λ

incubate at 42° for 2 minutes
Add 1λ SSII
Pipette gently up and down to mix
Put at 42° for 50 minutes
Move to 70° for 15 minutes
Quick spin
Add 80 λ dH_2O Keep @ -20 use 3.3λ per Real Time reaction