

## RT PCR of Embryos

### RT

RNA: 10 embryos snap frozen at various stages in liquid N<sub>2</sub>  
Prepare using RNeasy columns, elute in 50 uL RNase free DI H<sub>2</sub>O

#### 20 uL RT Reactions:

5 uL	RNA	) 13 uL total can vary amounts
8 uL	H <sub>2</sub> O	)
1 uL	oligo dT	(1.5 mg / mL)
4 uL	5 X RT Buffer	
0.33 uL	BSA	(3 mg / mL)
0.5 uL	dNTPs	(25 mM stock)
0.5 uL	RNasin	
0.5 uL	MMLV-RT	

Combine RNA, H<sub>2</sub>O, and Oligo-dT. Heat to 65C for 3 minutes.

Cool on ice, spin down briefly.

Add mix with remainder of ingredients.

Add 6 uL to each reaction.

Incubate at 42C for 30 min to 1 hour

(To remove contaminating DNA before starting, treat with DNase and repeat RNeasy column to cleanup)

#### 10 uL PCR Reactions:

4 uL	RT Reaction	
1 uL	10 X PCR buffer	
0.1 uL	dNTPs	(25 mM stock, add 1:50 32P-nucleotide)
0.5 uL	up Primer	(1 mg / mL)
0.5 uL	down Primer	(1 mg / mL)
3.8 uL	H <sub>2</sub> O	
0.1 uL	Taq	

(32P-CTP = 0.2 uL)

#### PCR machine steps:

94C	3 min
94C	1 min**
55C	1 min**
72C	1 min**
72C	5 min
4C	Hold

\*\* Repeat 15 – 25 X depending on template