Reverse Transcription (RT) of mRNA to cDNA

Purpose: To convert RNA samples (e.g., total RNA obtained by Trizol extraction of fetal tissues) to cDNA that is suitable to use as a template for PCR.

Reverse Transcription reagent	Product Name	Cat #	Company
Oligo-(dT) (–20 °C)	Oligo dT Primer	n420-01	Invitrogen
RT Kit (–20 °C):	SuperScript II RNase H- Reverse Transciptas	18064-014	Invitrogen
RT II Buffer 5x	RNase H- Reverse Transcriptase 5x Buffer		
RTII	SSII RT Reverse Transcriptase		
DTT	0.1M DTT		
RNAse Inhib. (–20 °C)	RNase Inhibitor	90435328	Roche
dNTPs (–20 °C)	Sequencing Grade Solutions dNTP's	27-2035-01	Amersham
RNAse H (–20 °C)	RNase H 100u	92125120	Roche

1. Prepare mRNA samples by mixing the following in 1.5 mL Eppendorf tubes (Up to 5 rxns may be done in one vial to generate sufficient amount of cDNA for stock soln.) $V_{tot/RNA\ prep}$ for 1 rxn=11µL, $V_{tot/RNA\ prep}$ 5 rxns = 55 µL

	Ci (ng/µL)	Cf (ng) per reaction	Cf (ng) for 5rxns	Vol. (uL) 5rxn
mRNA	1.5	2.5	12.5	8.33
Oligo-(dT)	12.5	25	125	10
ddH2O				36.67

2. Incubate samples for 10 min at 70 °C, and centrifuge. This destabilizes mRNA secondary structures. While samples are heating, prepare RT mixtures.

3. RT stock mix may be prepared in advance and stored at –20 °C.

RT Mix reagent	Ci	Cf	V/1 rxn (uL)	V/50 rxn (uL)
5x Buffer	5x	1x	4	200
DTT	.1 M	.01 M	2	100
dNTP	10mM ea.	.5mM	1	50
RNAse inhib.	40 U/µL	40 U	1	50

4. Add 40 µL of RT mix to each RNA prep.
5. Incubate 2min at 42°C
6. Add 5 µL of RT II (200 U/µL) to each vial (except RT- control vials)
7. Incubate for 60-90 min at 42 °C
8. Incubate for 10 min at 70 °C
9. Add 2.5 uL of RNAse H (1U/µL)
10. Incubate 15 min at 37 °C, centrifuge, and either use RT product immediately or store samples at –20 °C.

Notes:

1. Store RNA samples at –80 °C if necessary and handle carefully to prevent degradation. Once cDNA is prepared, the samples tend to be stable at –20 °C for extended periods.
2. RT- control (no RT II added to sample) checks for genomic DNA contamination of RNA samples.
3. To verify success of RT reaction, perform PCR with primers for a product that is known to be present in the sample (i.e. HPRT or GADPH)