

Genome Analysis Core UCSF Helen Diller Family Comprehensive Cancer Center			
Standard Operating Procedure			
Title: Reverse Transcription			
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**Introduction:**

Reverse transcription is the process in which cDNA is transcribed from RNA. DNase treatment is sometimes done beforehand to remove any genomic DNA from the RNA. This is especially necessary when looking at gene expression levels with primers and probes that are within a single exon.

**Additional Information:**

The Genome Core has been using the Bio-Rad iScript cDNA Synthesis Kit for at least two years, and has found it to be the best method in terms of ease, reliability, and use for a variety of applications. It uses a blend of oligo (dT) and random hexamer primers, thus working well with a wide variety of targets. The kit is optimized for targets < 1kb in length.

**In Each Reaction:**

Reagent (Initial Concentration)	Volume (µL)	Final Concentration
5x iScript Reaction Mix	4	1x
iScript Reverse Transcriptase	1	
Nuclease-free water	X	
RNA template (100fg to 1µg Total RNA)	X	
<b>Total</b>	<b>20 µL</b>	

**Reagents required:** (in the RT fridge)

- 5x iScript Reaction Buffer, iScript Reverse Transcriptase, and Nuclease Free H<sub>2</sub>O.
- RNA template with known concentration

**Basic Flow:**

- 1) Clean RNA Room and pipettman
- 2) Quantify RNA and perform necessary calculations
- 3) Make RT Master Mix
- 4) Pipette MM + H<sub>2</sub>O + RNA template to strip tube(s) = 20ul total/rxn
- 5) Run in thermocycler

**1. Clean RNA Room and Pipettman.**

- Wipe down the bench and pipettman with RNase ZAP.

**2. Quantify RNA and Perform Calculations.**

- Determine the concentration of the RNA samples using the nanodrop.

- Calculate the volume of RNA and H<sub>2</sub>O to be added to the RT rxn for EACH sample:  
**Volume RNA = 1000ng/ [RNA]**  
 \*If the calculated volume exceeds 15ul, try the calculation with 750ng or 500ng instead of 1000ng, so that the maximum volume of RNA, under 15ul, is used.  
**Volume H<sub>2</sub>O = 15ul – Volume RNA**
- This can easily be set up in an excel document with the exported nanodrop data:

Sample ID	[RNA] ng/ul	Vol. RNA 1000/[RNA]	Vol. H2O 15ul - Vol. RNA	[cDNA] ng/ul
1	455	2.20	12.80	50
2	585.04	1.71	13.29	50
...	...	...	...	...

### **3. Make RT Master Mix.**

- Calculate the number of reactions to make: # rxns = # samples + 1 or 2 extra.
- Multiply this number by each component of the RT Master Mix:

Reagents	1X	Example: 6rxns
5x iScript Buffer	4ul	24ul
iScript Reverse Transcriptase	1ul	6ul

- Add this volume of reagent to a 1.5ml tube, vortex, spin down and put on ice.

### **4. Pipette MM, H<sub>2</sub>O, and RNA Template to Strip Tube(s)**

- First add the calculated volume of H<sub>2</sub>O to each tube of a strip tube labeled by sample #.
- Next, add 5ul of RT Master Mix to each of the tubes.
- Finally, add the calculated volume of RNA to each of the corresponding tubes labeled with sample number.

### **5. Run Sample Reactions in Thermocycler**

- Run 'iScript' on the MJ: 25° for 5 min, 42° for 30 min, 85° for 5 min.