

REVERSE TRANSCRIPTION USING SUPERSCRIPT III AND OLIGO(DT)12-18**First Strand cDNA synthesis from RNA**

1. Added the following components to a thin-walled 0.6ml microfuge tube:

Component	Concentration	Amount added
Oligo(dt) ₁₂₋₁₈	500ng (200-500ng)	1.0ul
RNA	2ug (10pg-5ug)	x µl (Up to 11.0ul)
dNTP mix	10mM	1.0ul
Nuclease-free Water	N/A	Q.S to 13.0ul

2. Tap the mixture to mix and then quick spin to collect contents at bottom of the tube.
3. Heat the mixture to 65°C for 5 minutes and immediately incubate on ice for at least 1 minute
4. Tap the tube to collect any moisture that dispersed in the tube and then quick spin.
5. Add the following:

Component	Concentration	Amount Added
5x First-Strand Buffer	5x concentration	4.0ul
DTT	0.1M	1.0ul
RNase Out inhibitor	40U/ul	1.0ul
SuperScript III	200U/ul	1.0ul

6. Mix by pipeting up and down slowly then spin down contents
7. Incubate at 50°C for 60 minutes (30-60) and then 70°C for 15 minutes. Then hold at 4°C until ready, or freeze.
 - a. Thermal Cycler in 5S-11 uses program SS350
 - b. Thermal Cycler in 5S-19 uses program SSIII50
8. Store in the -80°C for long term storage and at 4°C for short term storage.