

DNase Treatment

RNA (5 ug)	X
10X DNase Buffer	5
DNase I	5
dH ₂ O	enough for 50 uL total reaction volume

Room Temp 15 min

Add 1 uL 25 mM EDTA

65 C 10 min

Reverse Transcriptase

Random Hexamers (250ng/uL)	5
DNased RNA (5ug)	50
dNTPs (10mM each)	5
dH ₂ O	5

65 C 5min

Chill on ice > 1min

Centrifuge

5X First Strand Bufer	20
0.1M DTT	5
RNAse out	5
Superscript III RT	5

Mix by genetly pipetting

25 C 5 min

50 C 60 min

70 C 15 min

4 C forever