0) Measure RNA amount			
1)	Mix and brief spin-down each reagent		
2)	Prepare following mixture in each PCR tube		
	RNA solution	x μL (up to 5 μg of to	otal RNA)
	dNTPs	1 μL	
	Oligo(dT)	1 μL	
	RNase-free water	8-x μL	
3)	Incubate 5 min at 65°C		
4)	Incubate at least 1 min at 4 °C		
5)	Prepare following reaction mixture in 1.5 mL tube		
	*Keep mixing order and 5 samples need indicated volume		
	1)	10×RT buffer	10 µL
	2)	$25 \mathrm{mM~MgCl}_2$	20 µL
	3)	0.1 M DTT	10 µL
	4)	RNase inhibitor	$5~\mu L$
6)	Add 9 µL of reaction mixture to each sample		
7)	Mix gently and brief spin-down		
8)	Incubate 2 min at 42 °C		
9)	Add 1 µL of SuperScript II RT enzyme		
10)	Mix <u>gently</u> and incubate 50 min at 42 °C		
11)	Incubate 15 min at 70 °C		
12)	Incubate at least 1 min at 4 °C		
13)	Brief spin-down and add 1 μ L of RNaseH		
14)	Mix <u>gently</u> and incubate 20 min at 37 °C		
15)	Measure DNA amount with NanoDrop		
16) Keep -20 °C			