Title	cDNA first strand synthesis		
Protocol Number	MOLBIO20004	Written by	Duha A.
Adapted from	MO Lab and VU & UGA and NEB	Version	1.0
Date created	19 June 2020	Version update	NA

Before starting:
- Use **1µg** of RNA for cDNA synthesis (you could use as high as 1µg or low as 10 pg in 20µL reaction)
- Enzymes need to be kept on ice

Reagents:
- Protoscript II Reverse Transcriptase (NEB #M0368)
- dNTPs (10mM)
- Random primers (invetrogen)
- 5X Protoscript II RT buffer
- 10X 0.1 M DTT
- RNAse H

Protocol:
1. Calculate the amount of RNA µL required to achieve 1µg (need to equal or be less than 8µL).
2. For each sample, prepare 2 eppendorfs with a mix of RNA+ dNTPs + random primers as follows:

Component	Volume (F_v = 10µL)	Volume (F_v = 20µL)
RNA	1-8 µL	9-16 µL
Random primers mix	1µL (of 60 µM conc)	2µL
dNTPs	1µL (10mM)	2µL
Nuclease free water	For a total volume of 10µL	For a total volume of 10µL

3. For each set of eppendorfs, Label one as 'sample' and the second as 'NC'
4. Incubate the eppendorfs at 65ºC for 5 mints to denature the primers
5. Spin sown the eppendorfs for 5 sec
6. Promptly place the eppendorfs on ice for at least 1 mint
7. To both eppendorfs add (except for **RT**: it is **ONLY** added to the sample tubes):

For this step, you can create 2 master mixes. One for the samples & the other for the negative controls. For the negative controls, replace RT with water

Component	Volume (F_v = 20µL)	Volume (F_v = 40µL)
5X ProtoScript II Buffer	4µL	8 µL
0.1 M DTT	2µL	4 µL
ProtoScript II RT (200 U/µl)	1µL	2 µL
Nuclease free water	3µL	6 µL

Component	**Volume (F_v = 20µL) for NC**	**Volume (F_v = 40µL) for NC**
5X ProtoScript II Buffer	4 µL	8 µL
0.1 M DTT	2µL	4 µL
ProtoScript II RT (200 U/µl)	NONE	NONE
Nuclease free water	4µL	8 µL

8. Since Random Primer Mix is used, an incubation step at 25°C for 5 minutes is recommended before the 42°C incubation.
9. Incubate the 20 µl cDNA synthesis reaction at 42-48°C for 1 hr.
10. Inactivate the enzyme by incubating at 65°C for 20 minutes.

RNA removal:

11. Add 1uL of *E.coli* RNAseH and incubate at 37 ºC for 20 minutes.
12. Inactivate RNAse H by heating at 65ºC for 10 minutes
13. Store the cDNA product at -20°C or proceed to RT-qPCR
14. You should not does the cDNA at this stage as the results will be falsely elevated due to the presence of enzymes, templates, primers, etc.