cDNA Synthesis using iScript cDNA synthesis kit

(protocol adapted from Biorad iScript cDNA synthesis kit Cat. No. 1708891)

Reagents and Equipment

- Biorad iScript cDNA synthesis kit Cat. No. 1708891
- Thermocycler

Protocol for macroareas

1. Vortex all components of the kit.
2. Mix RNA template without vortex.
3. Use the following reaction setup for *n*+1 samples:

Component	Vol/reaction
Nuclease-free water	x µl
5x iScript reaction mix	4 µl
iScript reverse transcriptase	1 µl
RNA template (100 fg to 1 µg total RNA)*	1-3 µl
Total Volume	20 µl

*When using larger amounts of input RNA (>1 µg), the reaction should be scaled up (for example, 40 µl reaction for 2 µg, or 100 µl reaction for 5 µg) to ensure optimum synthesis efficiency

4. Prepare a master mix for *n* samples in an eppendorf tube by adding (in this order) water, 5x iScript reaction mix, and iScript reverse transcriptase.
5. Vortex and distribute the mix by *n* PCR tubes.
6. Add RNA template to each PCR tube. Centrifuge if needed.
7. Use a thermocycler to incubate the reaction mix, using the following protocol:

Priming	5 min at 25 ºC
Reverse transcription	60 min at 42 ºC
RT inactivation	5 min at 85 ºC
Optional step	Hold at 4ºC

8. Store samples at -20 ºC.

Note: All tubes must be on ice and the reaction has to be made on ice or in a cold plate.

cDNA Synthesis using M-MLV Reverse Transcriptase

(protocol adapted from ThermoScientific Reverse Transcriptase Cat. No. 28025)

Reagents and Equipment

- M-MLV RT (200 U/µL) (Thermo Scientific Cat. no. 28025-013)
- Oligo (dT)12-18 (500 µg/mL) (Thermo Scientific Cat. no. SO132), or Random Hexamer Primer (Thermo Scientific Cat. no. SO142)
- dNTP Mix (10 mM) (Thermo Scientific Cat. no. R0192)
- RNaseOUT™ Recombinant Ribonuclease Inhibitor (Thermo Scientific Cat. no. 10777-019)
- UltraPure Distilled Water (Invitrogen Cat. No. 10977-015)
- Thermocycler

Protocol for macroareas

A 20-µL reaction volume can be used for 1 ng–5 µg of total RNA or 1–500 ng of mRNA.

9. Mix and briefly centrifuge all components after thawing, keep on ice.

10. Use the following reaction setup for *n*+1 samples:

Component	Vol/reaction
Distilled water	x µl
5X First Strand Buffer	4 µl
Oligo (dT)12-18 (500 µg/mL)	1.5 µl
10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP)	2 µl
RNase Inhibitor (40 units/µL)	0.5 µl
MMLV Reverse Transcriptase (200 units)	2 µl
Total RNA or mRNA	x µl
Total Volume	20 µl

Note: DTT, a reducing reagent, is often included for optimal enzyme activity but is optional. When using less than 50 ng of starting RNA, the addition of an RNase Inhibitor is essential.

11. Mix gently and distribute the mix by *n* PCR tubes.

12. Add RNA template to each PCR tube. Centrifuge if needed.

13. Use a thermocycler to incubate the reaction mix, using the following protocol:

a. If using oligo(dT) primer or gene-specific primers, incubate 60 min at 37ºC.
b. If using random hexamer primer, incubate 10 min at 25ºC followed by 60 min at 37ºC.
c. For the transcription of GC rich RNA reaction temperature can be increased to 45ºC.

Terminate the reaction by heating at 70ºC for 10 min. Do not heat-inactivate enzyme prior to analysis of long cDNA to avoid cleavage.

14. The reverse transcription reaction product can be directly used in PCR or stored at -20ºC.

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