cDNA synthesis

The following protocol is intended for the synthesis of full length first and second cDNA strands from pure RNA. The second strand synthesis is only required for transcript quantification in QPCR using a double stranded DNA standard.

Reagents and kits

1. Reverse Transcriptase: (Fermentas' RevertAid™ RT, Promega's ImProm-II™ RT, or Invitrogen's SuperScript™ III RT)
2. Random hexamer primers
3. RNase inhibitor: (Fermentas' Ribolock™, Promega's RNasin™, or Invitrogen's RNaseOUT™)
4. dNTP mix (10 mM)
5. BSA (20 µg/µl)

Procedure

1. Prepare the following mixture:

Template RNA	4 µl (10 ng - 5 µg)
Random primers (0.5 µg/µl) Or gene specific (2 pmole/µl)	1 µl
RNase free water	4 µl

2. Mix gently and spin down the solution.
3. Incubate the mixture at 70^{o} C for 5 min in a thermocycler and chill on ice.
4. Prepare the following mixture and add to each tube:

   a. Fermentas:

5x Reaction Buffer	4 µl
Ribolock™ (20 U/µl)*	1 µl
10 mM dNTP mix	2 µl
BSA (20 µg/µl)	1 µl
RevertAid™ RT (200 U/µl)	1 µl
RNase free water	2 µl

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b. Promega:

5x Reaction Buffer	4 µl
RNasin™ (40 U/µl)*	0.5 µl
$MgCl_2$ (25mM)	2.5 µl
10 mM dNTP mix	2 µl
BSA (20 µg/µl)	1 µl
ImProm-II™ RT (200 U/µl)	1 µl

c. Invitrogen:

5x Reaction Buffer	4 µl
10 mM dNTP mix	2 µl
0.1 M DTT*	1 µl
RNaseOUT™ (40 U/µl)*	1 µl
BSA (20 µg/µl)	1 µl
RNase free water	1 µl
SuperScript™ III RT (200 units/µl)	1 µl

* Optional

5. Incubate the mixture in a thermocycler at 25° C for 5 min (only for random primers) followed by 42° C* for 60 min and 70° C for 15 min. Chill on ice.

* 50° C in the case of SuperScript™ III RT (Invitrogen)

Second strand synthesis

1. Prepare the following mixture and add to each tube:

   a. Fermentas:

DNA Polymerase I reaction buffer	1 µl
DNA Polymerase I	0.75 µl
RNase H, *E.coli*	0.2 µl
DDW	3.05 µl
Template cDNA	5 µl

2. Incubate for at 15° C for 2 h followed by 75° C for 10 min for deactivation.
3. Purify the reaction by phenol/chloroform followed by ethanol precipitation or using a PCR purification kit.

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