cDNA synthesis using superscript II

by A. Untergasser (contact address and download at www.untergasser.de/lab) - Version: 1.0

ATTENTION: This is expensive. One reaction as described here is ca. 6 Euro! Check the quality of your RNA by gel to be sure it is not degraded.

1. Prepare the annealing mix:

RNA	50 ng/µl	1 µg
random hexamers (100 ng/µl)	25 ng/µl	5 µl
water		add to 20 µl

2. Anneal the primers in a thermocycler:

1.	70 °C	10 min
2.	25 °C	10 min
3.	4 °C	store

3. Prepare the enzyme mix:

5x First Strand Buffer	8 µl
DDT	4 µl
dNTP (10 mM each)	2 µl
SUPERase inhibitor	1 µl
SuperScript II	1 µl
water	4 µl

4. Prepare this mix as a master mix and add then 20 µl to each reaction.
5. Synthesize the cDNA in a thermocycler:

1.	25 °C	10 min
2.	37 °C	45 min
3.	42 °C	45 min
4.	70 °C	15 min
5.	4 °C	store

6. Dilute the reaction mix for use in qPCR
   Add 160 µl water to the 40 µl reaction mix.
   Use 2 µl per qPCR reaction (that's equivalent to 10 ng RNA).

Random Primer Hexamers:
Dilute 33.3 µl Random Primer Hexamers (3 µg/µl) with 966.7 µl water to obtain a 100 ng/µl solution.

Materials needed:
SuperScript II (# 18064-014) by Invitrogen
Random Primer Hexamers (3 µg / µl (# 48190-011) by Invitrogen
SUPERase Inhibitor (# 2694) by Ambion

Commented Protocol:
1. Prepare the annealing mix:

RNA	50 ng/µl	1 µg
random hexamers (100 ng/µl)	25 ng/µl	5 µl
water		add to 20 µl

Instead of the random hexamers you can use 4 pmole gene-specific primers or 1 µg Oligo(dT)$_{12-18}$ (each given as total amount for this reaction mix). I prefer the random hexamers, because one synthesis can be used for whatever you want to detect and is not enriching the 3' ends of the RNA. Furthermore, it just works well.

2. Anneal the primers in a thermocycler:

1.	70 °C	10 min
2.	25 °C	10 min
3.	4 °C	store

3. Prepare the enzyme mix:

5x First Strand Buffer	8 µl
DDT	4 µl
dNTP (10 mM each)	2 µl
SUPERase inhibitor	1 µl
SuperScript II	1 µl
water	4 µl

Prepare this mix as a master mix and add then 20 µl to each reaction.
This works really well. I make a big mix and add it to each tube as soon as the PCR is finished. I just do this at room temperature to avoid the trouble with ice.

4. Synthesize the cDNA:

Program	Temp.	Time
1.	25 °C	10 min
2.	37 °C	45 min
3.	42 °C	45 min
4.	70 °C	15 min
5.	4 °C	store

5. Dilute the reaction mix for use in qPCR

Add 160 µl water to the 40 µl reaction mix.
Use 2 µl per qPCR reaction (that's equivalent to 10 ng RNA).
For some applications it is necessary to get rid of the RNA. Use a RNase incubation followed by a clean up using spin columns.

Known Issues:

- The expensive stuff is the SuperScript enzyme. The rest is supplied in sufficient amounts. If you have less than 1 µg RNA, you can consider to reduce the volume to half and the enzyme to match the amount of RNA. Before you process hundreds of samples try one sample with your optimized protocol versus this standard version.

References and Comments:

This protocol has several roots. It is based on a protocol to create cDNA for microarrays and adapted to be used for lower amounts. It works very reliable and it is my preferred way of cDNA synthesis.

How to cite this page in publications:

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<http://www.untergasser.de/lab/protocols/cdna_synthesis_superscript_ii_v1_0.htm>.

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