Sep 01, 2020 Version 1

cDNA synthesis using SuperScript™ IV V.1

DOI
dx.doi.org/10.17504/protocols.io.qdnds5e

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.qdnds5e

Protocol Citation: Roey Angel, Eva Petrova 2020. cDNA synthesis using SuperScript™ IV. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.qdnds5e**

Protocol status: Working
We use this protocol and it's working

Created: May 24, 2018

Last Modified: September 01, 2020

Protocol Integer ID: 12430

Keywords: cDNA, RNA, mRNA, SuperScript, reverse transcriptase, superscript iv reverse transcriptase, using superscript iv reverse transcriptase, rna extract protocol, purification of rna, purified rna extract, cdna synthesis, rna extract, second strand synthesis, rna, cdna, dna, purification,

Abstract

The following protocol is intended as a downstream application for our **Purification of RNA from a DNA/RNA Extract** protocol. This protocol describes how to synthesise a first-strand non-specific complementary DNA (cDNA) from a purified RNA extract using **SuperScript IV Reverse Transcriptase**. The second strand synthesis is usually not required for most downstream applications. This protocol is a simplified and condensed version of the full protocol provided by the manufacturer.

Attachments

PDF

SSIV_First_Strand_Sy...

1.4MB

Materials

MATERIALS

- Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #**B14
- SuperScript™ IV First-Strand Synthesis System **Thermo Fisher Scientific Catalog #**18091050
- RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #**10777019
- Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #**18021071
- DNA Polymerase I (10 U/µL) **Thermo Fisher Scientific Catalog #**EP0041
- Random hexamers **Thermo Scientific Catalog #**N8080127

STEP MATERIALS

- Random hexamers **Thermo Scientific Catalog #**N8080127
- Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #**T143.1
- USB Dithiothreitol (DTT) 0.1M Solution **Thermo Fisher Scientific Catalog #**707265ML
- RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #**10777019
- SuperScript™ IV First-Strand Synthesis System **Thermo Fisher Scientific Catalog #**18091050
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Troubleshooting

Before start

Make sure your RNA is pure and contains no traces of DNA. A simple and very sensitive way to ensure that is to use the purified RNA as a template for a PCR reaction targeting a gene that should be present in the sample. A negative result indicates a lack of DNA template in the sample.

Primer annealing

1 Prepare the following mixture in a PCR tube:

1. 1 µL to 4 µL purified RNA (10 pg - 5 µg ; usually 200 ng for soil extract)
2. 1 µL random hexamers (50 µM) or a gene-specific primer (2 µM)
3. 9.8 µL RNase free water

Random hexamers **Thermo Scientific Catalog #**N8080127

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #**T143.1

2 Mix gently and spin down the solution.

3 Incubate the mixture at 65 °C for 00:05:00 in a thermocycler and chill On ice (or in the cycler at > 4 °C) for at least 00:01:00 .

Reverse transcription

4 Prepare the following mixture and add to each tube:

1. 4 µL 5x Reaction buffer
2. 1 µL dNTP mix, 10 mM
3. 1 µL 0.1M DTT
4. 1 µL RNaseOUT™ (40 U/µl) *
5. 0.2 µL BSA (20 µg/µl)
6. 1 µL SuperScript™ IV RT (200 units/µl)

* Optional

USB Dithiothreitol (DTT) 0.1M Solution **Thermo Fisher Scientific Catalog #**707265ML

SuperScript™ IV First-Strand Synthesis System **Thermo Fisher Scientific Catalog #**18091050

RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #**10777019

Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #**B14

5 Incubate the mixture in a thermocycler at 23 °C for 00:10:00 (only if using random hexamers, skip if using a specific primer) followed by 50 °C for 01:00:00 to 03:00:00 and 80 °C for 00:10:00. Chill On ice.

6 **For PCR templates > 1kb** remove the RNA by adding 1 µL (2 units) of E. coli RNase H and incubate at 37 °C for 00:20:00.

Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #**18021071

Optional: Second strand synthesis

7 Prepare the following mixture and add to each tube:

1. 1 µL DNA Polymerase I reaction buffer
2. 0.75 µL DNA Polymerase I
3. 0.2 µL RNase H
4. 3.05 µL RNase-free water
5. 5 µL Template cDNA

DNA Polymerase I (10 U/µL) **Thermo Fisher Scientific Catalog #**EP0041

Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #**18021071

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #**T143.1

8 Incubate for at 15 °C for 02:00:00 followed by 00:10:00 at 75 °C for deactivation.

9 3. Purify the reaction through phenol/chloroform purification followed by ethanol precipitation or using a PCR purification kit.