VERSION 1

SEP 01, 2020

OPEN ACCESS

DOI:
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>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: May 24, 2018

Last Modified: Sep 01, 2020

PROTOCOL integer ID:
 12430

cDNA synthesis using SuperScript™ IV V.1

Roey Angel¹, Eva Petrova¹

¹Soil and Water Research Infrastructure

SoWa RI Anaerobic and Molecular Microbiology (public)
 Tech. support email: eva.petrova@bc.cas.cz

Roey Angel

Soil and Water Research Infrastructure

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

[SSIV_First_Strand_Synthesis_System_UG.pdf](#)

Keywords: cDNA, RNA, mRNA, SuperScript, reverse transcriptase

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

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

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

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

BEFORE START INSTRUCTIONS

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 $^{\circ}\text{C}$ for 00:05:00 in a thermocycler and chill On ice (or in the cycler at > 4 $^{\circ}\text{C}$) 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.