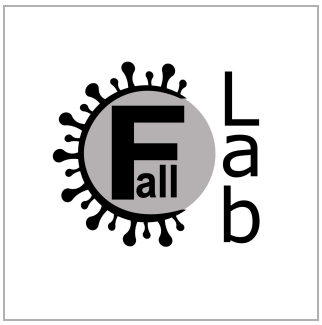

DEC 12, 2022

WORKS FOR ME 1

Synthesis of double-strand cDNA (ds-cDNA) from viral dsRNA by using Random primers

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.81wgbyrxnvpk/v1

Vahid Jalali Javaran[1]

[1]Département de Biologie, Centre SÈVE, Université de Sherbrooke, Sherbrooke, QC J1K 2R1, Canada

Nanovirseq

Vahid Jalali Javaran

ABSTRACT

Double-stranded cDNA synthesis from viral dsRNAs:
For dsRNA sequencing by nanopore sequencing, this protocol was used. Before treating samples with RNase T1, you should measure the total concentration of RNAs in the samples by using a nanodrop or Qubit device, as RNase T1 has the ability to partially digest double-stranded RNAs in the absence of single-stranded RNA.

DOI

dx.doi.org/10.17504/protocols.io.81wgbyrxnvpk/v1

PROTOCOL CITATION

Vahid Jalali Javaran 2022. Synthesis of double-strand cDNA (ds-cDNA) from viral dsRNA by using Random primers. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbyrxnvpk/v1

CREATED

Dec 12, 2022

LAST MODIFIED

Dec 12, 2022

PROTOCOL INTEGER ID

73881

MATERIALS TEXT

RNase T1 and DNase I digestion

- DNase I (RNase-free)
- DNase I Reaction Buffer with MgCl2 (10X)
- RNase T1

Synthesize the first strand of cDNA (Reverse Transcription)

- Random primers (60 µM)
- dNTP (10 mM)
- H_2O
- First Strand cDNA synthesis Buffer
- RNase out or RNasin® Ribonuclease Inhibitor (40 u/µl)
- Superscript III or Maxima H minus

Removal of the residual RNA by RNase H

- RNase H

Synthesize the second strand of cDNA

- Klenow 10X buffer+NAD,
- dNTP (10 mM),
- Klenow DNA Polymerase I,
- E. coli DNA Ligase I

cDNA purification by AMPure XP beads

- Agencourt AMPure XP
- Magnet plate
- Ethanol 80%
- Nuclease free water
- 1.5 ml eppendorf tube

1h 20m

RNase T1 and DNase I digestion

20m

1

- Add 10X DNase Buffer with MgCl2 (final concentration should be 1X).
- Add 50 units RNase T1 per 1µg of total RNA and 1 unit DNase I per 2ug of total RNA.
- Incubate at 37 degrees C for 20 min.

1h

1.1 **Stop reaction**

Do a phenol-chloroform extraction and Ethanol precipitation of dsRNA.

Citation: Vahid Jalali Javaran Synthesis of double-strand cDNA (ds-cDNA) from viral dsRNA by using Random primers https://dx.doi.org/10.17504/protocols.io.81wgbyrxnvpk/v1

Synthesis of the first strand of cDNA

5m

2 Mix well below components by pipetting and centrifuge or spin briefly.

A	B
Treated dsRNA	5 µl
Random primers (60 µM)	2µl
dNTP (10 mM)	1 µl
H2O	6 µl
Total	14 µl

- Incubate at 99C for 5 mins and put tubes **immediately on water ice** (it is better to do this step with a thermocycler).

2.1 Add below components and mix well by pipetting and centrifuge or spin briefly.

A	B
First Strand Buffer	4 µl
RNase out or RNasin® Ribonuclease Inhibitor (40 u/µl)	1 µl
Maxima H minus (Point: just use 200 unit)	1 µl
Total	20 µl

1h 55m

2.2 Incubation step.

A	B
25 C	20 mins
55 C	90 mins
85 C (Inactivation step)	5 mins

20m

Removal of residual RNA by RNase H

20m

3 Mix well below components by pipetting and centrifuge or spin briefly.

A	B
cDNA	20 µl
RNase H	1 µl

Citation: Vahid Jalali Javaran Synthesis of double-strand cDNA (ds-cDNA) from viral dsRNA by using Random primers https://dx.doi.org/10.17504/protocols.io.81wgbyrxnvpk/v1

A	B
Total	21 µl

Incubate at 37 C for 20 mins

2h 40m

Synthesis of the second strand of cDNA

4 Mix well below components by pipetting and centrifuge or spin briefly.

A	B
cDNA	21 µl
Klenow 10X buffer	2.7 µl
dNTP (10 mM)	1 µl
Klenow DNA Polymerase I	0.7 µl
E. coli DNA Ligase I	1 µl
H2O	0.6 µl
Total	27 µl

2h 40m

4.1 Incubation step.

A	B
16 C	150 mi
75 C; (Inactivation step) Point: check this part based on enzyme brand.	10 min

17m

cDNA purification by AMPure XP

7m

5 Mix well below components by pipetting and incubate at room temperature for 5 mins.
Point: Put bead stock in room temperature for 20 min (before using). Vortex the bead stock very well (before using)

A	B
double stranded-cDNA	27 µL
AMPure XP	49 µL

Place the reaction tube onto a magnet plate for 2-5 mins
IMPORTANT: Wait for the solution to clear before proceeding to the next step.

5.1 This step must be performed while the reaction tube is situated on the magnet plate:

- Aspirate the cleared solution from the reaction tube and discard. Leave 5 µL of supernatant

Citation: Vahid Jalali Javaran Synthesis of double-strand cDNA (ds-cDNA) from viral dsRNA by using Random primers https://dx.doi.org/10.17504/protocols.io.81wgbyrxnvpk/v1

behind, otherwise, beads are drawn out with the supernatant.

IMPORTANT: Do not disturb the ring of separated magnetic beads.

1m

5.2 This step must be performed while the reaction tube is situated on the magnet plate:
Dispense 200 µL of 80% ethanol to the reaction tube and incubate for 30 seconds at room temperature.
Aspirate out the ethanol and discard.
Repeat previous step.

2m

5.3 After removing and discarding all supernatant, **A dry time is optional (2 to 5 mins)** to ensure all traces of Ethanol are removed.
NOTE: **For fragments 10 kb and larger, do not over-dry the bead ring** (bead ring appears cracked if over dried) as this will significantly decrease elution efficiency.

2m

5.4 Remove the reaction tube from the magnet plate, and then add 40 µL of nuclease-free water to the reaction tube and pipette mix 10 times. Incubate for 2 minutes.
NOTE The liquid level will be high enough to contact the magnetic beads at a 40 µL nuclease-free water. A greater volume of nuclease-free water can be used, but using less than 40 µL will require extra mixing (to ensure the liquid comes into contact with the beads), and may not be sufficient to elute all cDNAs.

5m

5.5 Place the reaction tube onto the magnet plate for 5 minute to separate beads from the solution.
IMPORTANT Wait for the solution to clear before proceeding to the next step.
Transfer the eluate to a new plate.

Use 1 µL of eluted ds-cDNA for concentration measurement by Qubit.

Citation: Vahid Jalali Javaran Synthesis of double-strand cDNA (ds-cDNA) from viral dsRNA by using Random primers
https://dx.doi.org/10.17504/protocols.io.81wgbyrxnvpk/v1