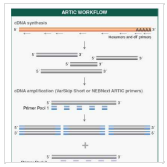


Sep 24, 2021

NEBNext® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626

Forked from NEBNext® Varskip Short ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®) E7658 Express Protocol with One Clean-up Step

New England Biolabs[1]

[1]New England Biolabs

dx.doi.org/10.17504/protocols.io.byfdpti6

New England Biolabs (NEB) Coronavirus Method Development Community

Isabel Gautreau
New England Biolabs

ABSTRACT

This protocol details methods for the NEBNext® ARTIC SARS-CoV-2 RT-PCR Module, NEB #E7626S/L 24/96 reactions.

cDNA Synthesis and Targeted cDNA Amplification with NEBNext VarSkip Short Primer Mixes:
This protocol follows an alternate variant-tolerant approach for targeting SARS-CoV-2 by utilizing NEBNext VarSkip Short SARS-CoV-2 Primer Mixes. The NEBNext VarSkip Short SARS-CoV-2 Primer mixes cannot be added to the same cDNA amplification reaction as the NEBNext ARTIC SARS-CoV-2 Primer Mixes. If downstream applications include sequencing, performing RNA input normalization prior to cDNA synthesis and targeted amplification promotes more even distribution of reads across sequencing libraries.

For other NEBNext® ARTIC SARS-CoV-2 protocols, please see the NEBNext ARTIC Protocols Collection.

To obtain instructions for using NEBNext ARTIC SARS-CoV-2 Primer Mix and the NEBNext® ARTIC SARS-CoV-2 RT-PCR Module please see the NEBNext ARTIC SARS CoV2 RT PCR Module Manual.

DOI

dx.doi.org/10.17504/protocols.io.byfdpti6

EXTERNAL LINK

https://www.neb.com/products/e7626-nebnext-artic-sars-cov-2-rt-pcr-module#Product%20Information

PROTOCOL CITATION

New England Biolabs 2021. NEBNext® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626. **protocols.io** https://dx.doi.org/10.17504/protocols.io.byfdpti6

FORK NOTE

FORK FROM

Forked from NEBNext® Varskip Short ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®) E7658 Express Protocol with One Clean-up Step , Isabel Gautreau

KEYWORDS

NEBNext, NEB, ARTIC, SARS-CoV-2, Library Prep, Illumina

Citation: New England Biolabs (09/24/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626. https://dx.doi.org/10.17504/protocols.io.byfdpti6

CREATED

Sep 21, 2021

LAST MODIFIED

Sep 24, 2021

PROTOCOL INTEGER ID

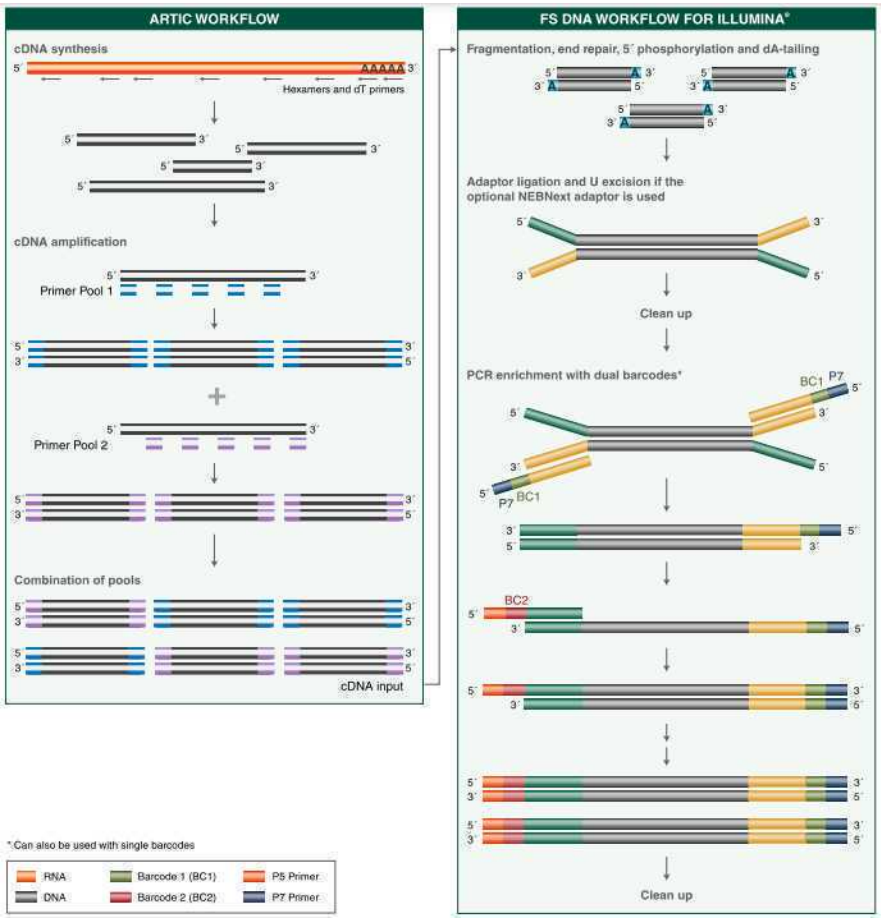
53445

GUIDELINES

Overview

The NEBNext ARTIC SARS-CoV-2 RT-PCR Module contains the enzymes and buffers required to convert a broad range of total RNA into high quality, targeted cDNA amplicons with minimal hands-on time.

The NEBNext VarSkip Short SARS-CoV-2 Primer Mixes included provide an alternate variant-tolerant approach for targeting SARS-CoV-2. The NEBNext VarSkip Short SARS-CoV-2 Primer Mixes cannot be added to the same cDNA amplification reaction as the NEBNext ARTIC SARS-CoV-2 Primer Mixes.



Workflow demonstrating the use of NEBNext ARTIC SARS-CoV-2 RT-PCR Module.

MATERIALS TEXT

Citation: New England Biolabs (09/24/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626. https://dx.doi.org/10.17504/protocols.io.byfdpti6

The Library Kit Includes
The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7658S) and 96 reactions (NEB #E7658L).

Store at –20°C.
(lilac) LunaScript® RT SuperMix
(lilac) Q5® Hot Start High-Fidelity 2X Master Mix
(Orange) NEBNext VarSkip Short SARS-CoV-2 Primer Mix 1
(Orange)NEBNext VarSkip Short SARS-CoV-2 Primer Mix 2
(lilac) NEBNext ARTIC SARS-CoV-2 Primer Mix 1
(lilac) NEBNext ARTIC SARS-CoV-2 Primer Mix 2
(white) 0.1X TE Buffer
(white) Nuclease-free Water

Required Materials Not Included

- 80% Ethanol (freshly prepared)
- DNA LoBind Tubes (Eppendorf® #022431021)
- Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific, Inc.® Q32851)
- Magnetic rack/stand (NEB #S1515S; Alpaqua®, cat. #A001322 or equivalent)
- Thermal cycler
- Vortex Mixer
- Microcentrifuge
- Agilent® Bioanalyzer® or similar fragment analyzer and associated consumables
- DNase RNase free PCR strip tubes (USA Scientific 1402-1708)

Kit Components
NEB #E7658S Table of Components

A	B	C
NEB #	**PRODUCT**	**VOLUME**
E7651A	LunaScript RT SuperMix	0.048 ml
E7652A	Q5 Hot Start High-Fidelity 2X Master Mix	0.3 ml
E7725A	NEBNext ARTIC SARS-CoV-2 Primer Mix 1	0.042 ml
E7726A	NEBNext ARTIC SARS-CoV-2 Primer Mix 2	0.042 ml
E8005A	NEBNext VarSkip Short SARS-CoV-2 Primer Mix 1	0.042 ml
E8006A	NEBNext VarSkip Short SARS-CoV-2 Primer Mix 2	0.042 ml
E7657A	0.1X TE	1.3 ml
E7667A	Nuclease free-Water	1.5 ml

NEB #E7658L Table of Components

A	B	C
NEB #	**PRODUCT**	**VOLUME**
E7651AA	LunaScript RT SuperMix	0.192 ml
E7652AA	Q5 Hot Start High-Fidelity 2X Master Mix	1.2 ml
E7725AA	NEBNext ARTIC SARS-CoV-2 Primer Mix 1	0.168 ml
E7726AA	NEBNext ARTIC SARS-CoV-2 Primer Mix 2	0.168 ml
E8005AA	NEBNext VarSkip Short Primer Mix 1	0.168 ml
E8006AA	NEBNext VarSkip Short Primer Mix 2	0.168 ml
E7657AA	0.1X TE	5.2 ml
E7667AA	Nuclease free-Water	1.5 ml

Companion Products

Citation: New England Biolabs (09/24/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626. https://dx.doi.org/10.17504/protocols.io.byfdpti6

A	B	C
NEB #	**PRODUCT**	**VOLUME**
T2010S	Monarch® Total RNA Miniprep Kit	50 preps

NEBNext ARTIC SARS-CoV-2 Primers
NEBNext ARTIC SARS-CoV-2 Primers for SARS-CoV-2 genome amplification are based on hCoV-2019/nCoV-2019 Version 3 (v3) sequences with balanced primer concentrations. Sequence information can be found at: https://github.com/joshquick/artic-ncov2019/blob/master/primer_schemes/nCoV-2019/V3/nCoV-2019.tsv

NEBNext VarSkip Short SARS-CoV-2 Primer Mix 1 and 2
NEBNext VarSkip Short SARS-CoV-2 Mix 1 and 2 for SARS-CoV-2 genome amplification were designed to reduce the impact of variants on amplification efficiency. Sequence information can be found at: https://github.com/nebiolabs/VarSkip

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

BEFORE STARTING

The amount of RNA required for detection depends on the abundance of the RNA of interest. In general, we recommend, using > 10 copies of the SARS-CoV-2 viral genome as input. In addition, we recommend setting up a no template control reaction and that reactions are set-up in a hood.

The presence of carry-over products can interfere with sequencing accuracy, particularly for low copy targets. Therefore, it is important to carry out the appropriate no template control (NTC) reactions to demonstrate that positive reactions are meaningful.

cDNA Synthesis

1

Gently mix and spin down the LunaScript RT SuperMix reagent. Prepare the cDNA synthesis reaction as described below:

A	B
COMPONENT	VOLUME
RNA Sample	8 µl
(lilac) LunaScript RT SuperMix	2 µl
Total Volume	10 µl

For no template controls, mix the following components:

A	B
COMPONENT	VOLUME
(white) Nuclease-free Water	8 µl
(lilac) LunaScript RT SuperMix	2 µl
Total Volume	10 µl

2

Incubate reactions in a thermocycler* with the following steps:

Citation: New England Biolabs (09/24/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626. https://dx.doi.org/10.17504/protocols.io.byfdpti6

A	B	C	D
CYCLE STEP	TEMP	TIME	CYCLES
Primer Annealing	25°C	2 minutes	1
cDNA Synthesis	55°C	20 minutes	
Heat Inactivation	95°C	1 minute	
Hold	4°C	∞	

*Set heated lid to 105°C

Samples can be stored at -20 °C for up to a week.

cDNA Amplification

3

4.5 µl of cDNA is recommended. If using less than 4.5 µl of cDNA, add nuclease-free water to a final volume of 4.5 µl. We recommend setting up the cDNA synthesis and cDNA amplification reactions in different rooms to minimize cross-contamination of future reactions.

NEBNext VarSkip Short Primer Mixes cannot be combined with NEBNext ARTIC Primer Mixes in the same targeted amplification reaction.

Gently mix and spin down reagents. Prepare the split pool cDNA amplification reactions as described below:

For Pool Set A:

A	B
COMPONENT	VOLUME
cDNA (Previous Step)	4.5 µl
(lilac) Q5 Hot Start High-Fidelity 2X Master Mix	6.25 µl
NEBNext VarSkip Short SARS-CoV-2 Primer Mix 1	1.75 µl
Total Volume	12.5 µl

For Pool Set B:

A	B
COMPONENT	VOLUME
cDNA (Step 2)	4.5 µl
(lilac) Q5 Hot Start High-Fidelity 2X Master Mix	6.25 µl
NEBNext VarSkip Short SARS-CoV-2 Primer Mix 2	1.75 µl
Total Volume	12.5 µl

4

Citation: New England Biolabs (09/24/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626. https://dx.doi.org/10.17504/protocols.io.byfdpti6

Incubate reactions in a thermocycler* with the following steps:

A	B	C	D
CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denature	95°C	15 seconds	35
Annealing/Extension	63°C	5 minutes	
Hold	4°C	∞	1

*Set heated lid to 105°C

5 Combine the Pool A and Pool B PCR reactions for each sample.

Samples can be stored at –20°C for up to a week.

Please consider the downstream application before proceeding. The next section lists instructions for a standard bead cleanup, however, it may not be necessary for your application.

Cleanup of cDNA Amplicons 7m

6

The volume of NEBNext Sample Purification Beads provided here are for use with the sample composition at this step (25 µl; Step 5). These bead volumes may not work properly for a cleanup at a different step in the workflow, or if this is a second cleanup at this step. For cleanups of samples contained in different buffer conditions, the volumes may need to be experimentally determined.

Vortex NEBNext Sample Purification Beads to resuspend.

7

Add **20 µl (0.8 X) resuspended beads** to the PCR reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

8 5m

Incubate samples on bench top for at least **00:05:00** at **Room temperature** .

9 Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant.

10 After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

Caution: do not discard the beads.

Citation: New England Biolabs (09/24/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626. https://dx.doi.org/10.17504/protocols.io.byfdpti6

11

Add **200 µl 80% freshly prepared ethanol** to the tube/plate while in the magnetic stand. Incubate at **Room temperature** for **00:00:30** , and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

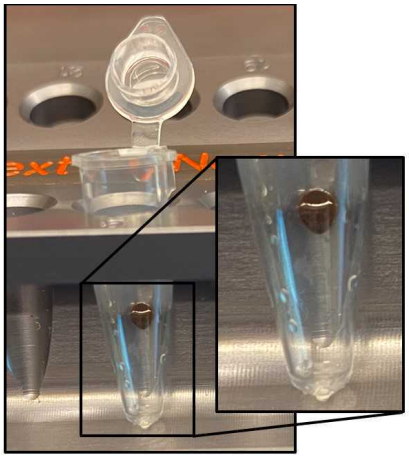
12

Repeat the previous step once for a total of two washes:
Add **200 µl 80% freshly prepared ethanol** to the tube/plate while in the magnetic stand. Incubate at **Room temperature** for **00:00:30** , and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

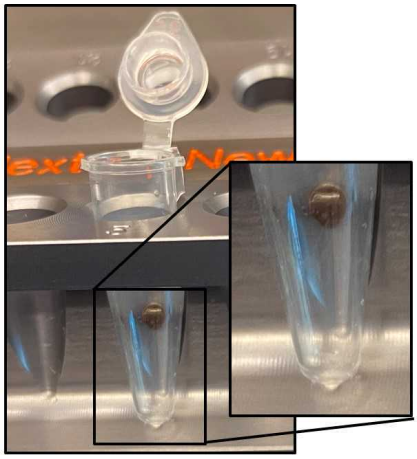
Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

13 Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

Caution: Do not over-dry the beads. This may result in lower recovery of DNA. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack, they are too dry.



After ethanol is removed the beads will be shiny and droplets of ethanol will be on the inside of the tube



When the beads are ready to elute visible droplets are gone and the beads are still dark brown and look a little matte

14

Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding **16 µl 0.1X TE** . Elution volume can be adjusted for specific applications.

15 2m

Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least **00:02:00** at

Citation: New England Biolabs (09/24/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626. https://dx.doi.org/10.17504/protocols.io.byfdpti6

Room temperature . If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

16

Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer **14 µl** to a new PCR tube. If elution volume was adjusted, the transfer volume should also be adjusted.

17 We recommend assessing the cDNA amplicon concentrations with a size distribution with Qubit® fluorometer.

Samples can be stored at –20°C for up to a week.

Amplicons may also be run on a Bioanalyzer or TapeStation® to confirm ~550 bp size of amplicons. To run on a TapeStation, dilute amplicon 10-fold with 0.1X TE Buffer and run 2 µl on a DNA High Sensitivity ScreenTape®. (See Figure 17 below for example of amplicon size profile on a Bioanalyzer).

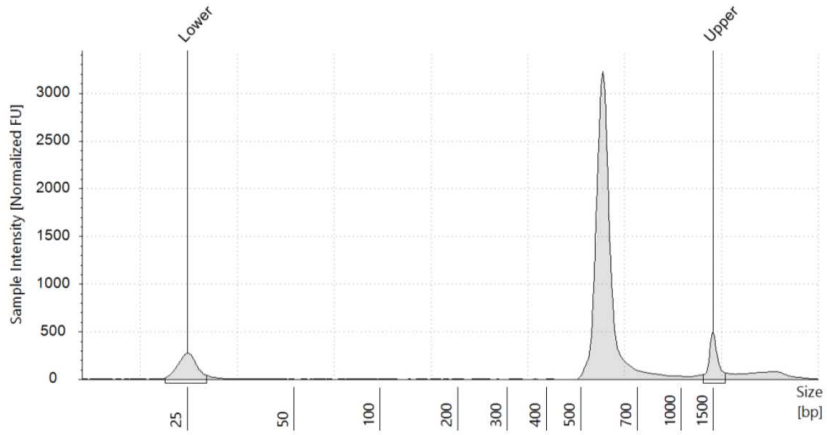


Figure 17 VarSkip Short SARS-CoV-2 cDNA amplicons generated from 1,000 total viral copies.

Citation: New England Biolabs (09/24/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 RT-PCR Module E7626. https://dx.doi.org/10.17504/protocols.io.byfdpti6