Sep 20, 2021

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

Forked from NEBNext® ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®) E7658 Express Protocol without PCR Bead Cleanup

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dx.doi.org/10.17504/protocols.io.bx9apr2e

New England Biolabs (NEB) Coronavirus Method Development Community

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ABSTRACT

This protocol details methods for the NEBNext® ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®), NEB #E7658S/L 24/96 reactions.

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. This protocol does not include a cleanup step for each sample after cDNA synthesis and after adaptor ligation. Performing RNA input normalization prior to cDNA synthesis and targeted amplification and/or normalizing final libraries prior to sequencing promotes more even distribution of reads across libraries. Skipping RNA input normalization, final library normalization, and cleanups reduces hands on time but may require deeper sequencing depth to reach sufficient coverage of each sample.

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

DOI

dx.doi.org/10.17504/protocols.io.bx9apr2e

EXTERNAL LINK

https://www.neb.com/-/media/nebus/files/manuals/manuale7658.pdf?rev=7253d569aa3040069cb09a9aa3724f2d&hash=53C7EDF0D2FDEDAE75308379A5EB2D89

PROTOCOL CITATION

New England Biolabs 2021. NEBNext® Varskip Short ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®) E7658 Express Protocol with One Clean-up Step . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bx9apr2e

FORK NOTE

FORK FROM

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KEYWORDS

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

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GUIDELINES

Overview

The NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit for Illumina contains the enzymes, buffers and oligos required to convert a broad range of total RNA into high quality, targeted, libraries for next-generation sequencing on the Illumina platform. Primers targeting the human EDF1 (NEBNext ARTIC Human Primer Mix 1) and NEDD8 (NEBNext ARTIC Human Primer Mix 2) genes are supplied as optional internal controls. The fast, user-friendly workflow also has minimal hands-on time.

Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together by construction and sequencing of indexed libraries on an Illumina sequencing platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Figure 1. Workflow demonstrating the use of NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit.

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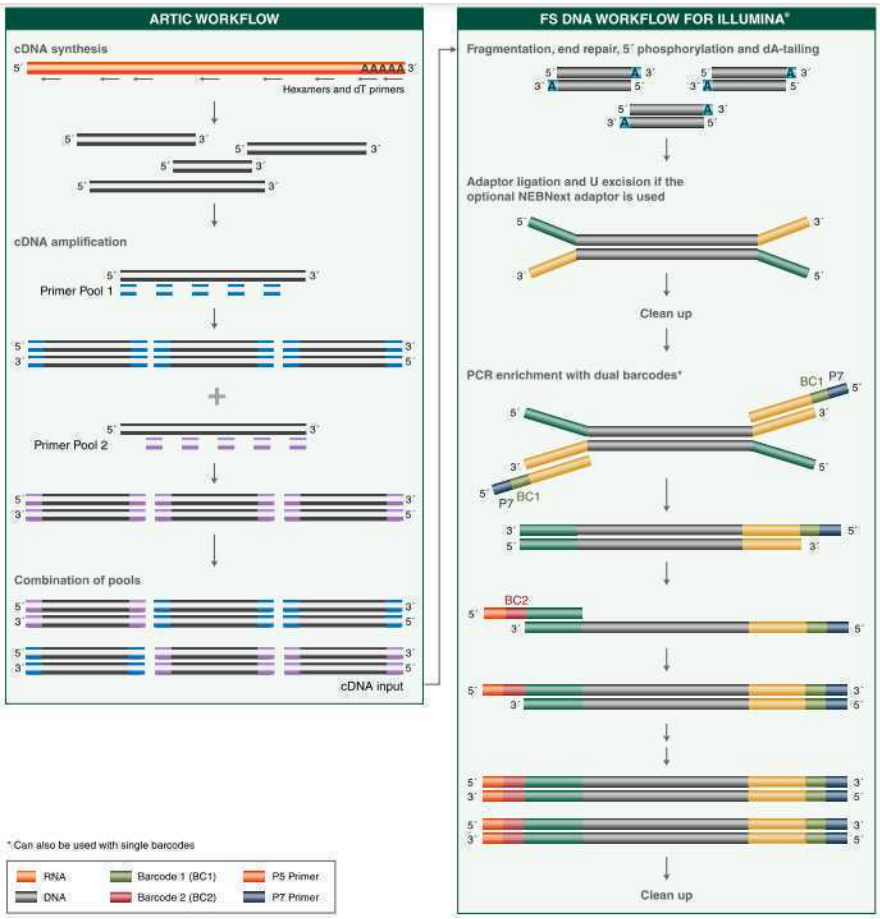

MATERIALS TEXT

The Library Kit Includes

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

Package 1: 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
(lilac) NEBNext ARTIC Human Primer Mix 1
(lilac) NEBNext ARTIC Human Primer Mix 2
(yellow) NEBNext Ultra II FS Enzyme Mix
(yellow) NEBNext Ultra II FS Reaction Buffer
(red) NEBNext Ultra II Ligation Master Mix
(blue) NEBNext Library PCR Master Mix
(white) 0.1X TE Buffer
(white) Nuclease-free Water

Package 2: Store at room temperature. Do not freeze.

NEBNext Sample Purification Beads

Required Materials Not Included

- 80% Ethanol (freshly prepared)
- DNA LoBind Tubes (Eppendorf® #022431021)
- Oxford Nanopore Technologies Native Barcoding Expansion kits 1-12 (EXP-NBD104) and 13-24 (EXP-NBD114)

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- Oxford Nanopore Technologies Ligation Sequencing Kit (SQK-LSK109)
- Oxford Nanopore Technologies SFB Expansion Kit (EXP-SFB001)
- 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 (#4150 or #4200 TapeStation System)
- DNase RNase free PCR strip tubes (USA Scientific 1402-1708)
- 1.5 ml tube magnet stand (NEB #S1506)

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
E7727A	NEBNext ARTIC Human Primer Mix 1	0.010 ml
E7728A	NEBNext ARTIC Human Primer Mix 2	0.010 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
E7668A	NEBNext Ultra II FS Enzyme Mix	0.024 ml
E7669A	NEBNext Ultra II FS Reaction Buffer	0.084 ml
E7655A	NEBNext Ultra II Ligation Master Mix	0.36 ml
E7656A	NEBNext Library PCR Master Mix	0.3 ml
E7657A	0.1X TE	1.3 ml
E7667A	Nuclease free-Water	1.5 ml
E7659S	NEBNext Sample Purification Beads	2.1 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
E7727AA	NEBNext ARTIC Human Primer Mix 1	0.034 ml
E7728AA	NEBNext ARTIC Human Primer Mix 2	0.034 ml
E8005AA	NEBNext VarSkip Short Primer Mix 1	0.168 ml
E8006AA	NEBNext VarSkip Short Primer Mix 2	0.168 ml
E7668AA	NEBNext Ultra II FS Enzyme Mix	0.096 ml
E7669AA	NEBNext Ultra II FS Reaction Buffer	0.336 ml
E7655AA	NEBNext Ultra II Ligation Master Mix	2 x 0.72 ml
E7656AA	NEBNext Library PCR Master Mix	1.2 ml
E7657AA	0.1X TE	5.2 ml
E7667AA	Nuclease free-Water	1.5 ml
E7659L	NEBNext Sample Purification Beads	4 x 2.1 ml

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Companion Products

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

NEBNext ARTIC Human Primers

A	B	C	D
PRIMER MIX	GENE	POSITION	PRIMERS
NEBNext ARTIC Human Primer Mix 1	EDF1	113 bp – 501 bp	GGCCAAATCCAAGCAGGCTA GTGTTCATTTCGCCCTAGGC
NEBNext ARTIC Human Primer Mix 2	NEDD8	110 bp – 489 bp	AAAGTGAAGACGCTGACCGG GGGATCCTCACAGTCTCCCA

Detailed information for the ARTIC Human control primers can be found at: https://doi.org/10.5281/zenodo.4495958

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

Note: We recommend using the express protocol for inputs of ≥ 100 copies of the (SARS-CoV-2) viral genome. The use of lower input amounts may result in significant levels of adaptor dimer in the sequencing data. In addition, we recommend setting up a no template control reaction. It is advisable to set up your reactions in the 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.

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

To obtain instructions for using NEBNext VarSkip Short SARS-CoV-2 Primer Mix and the NEBNext® ARTIC SARS-CoV-2 FS for Illumina STANDARD workflow (with cleanups) please contact NEB using info@neb.com.

cDNA Synthesis

1

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

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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:

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 cDNA input 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.

Use of the NEBNext ARTIC Human Primer Mix 1 and 2 are optional. If used, the appropriate NEBNext ARTIC Human Primer Mix and NEBNext VarSkip Short SARS-Cov-2 Primer Mix should be combined prior to use. More specifically, NEBNext ARTIC Human Primer Mix 1 should be combined with NEBNext VarSkip Short SARS-CoV-2 Primer Mix 1 and NEBNext ARTIC Human Primer Mix 2 with NEBNext VarSkip Short SARS-CoV-2 Primer Mix 2. Mixing directions are listed below.

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4

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

For Pool Set A:
If using the NEBNext ARTIC Human Primer Mix and a *24 reaction kit*, combine

0.7 µl NEBNext ARTIC Human Primer Mix 1 with

42 µl NEBNext VarSkip Short SARS-CoV-2 Primer Mix 1 in a new tube, vortex and spin down reagents. If using a *96 reaction kit*, combine **2.8 µl NEBNext ARTIC Human Primer Mix 1** with

168 µl NEBNext VarSkip Short SARS-CoV-2 Primer Mix 1 in a new tube, vortex and spin down reagents.

Use **1.75 µl combined mix** for each Pool Set A reaction.

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 1 *	1.75 µl
Total Volume	12.5 µl

* If using NEBNext ARTIC Human Primer Mix 1, add 1.75 µl of the combined NEBNext VarSkip Short SARS-CoV-2 Primer Mix 1 and NEBNext ARTIC Human Primer Mix 1.

For Pool Set B:
If using the NEBNext ARTIC Human Primer Mix and a *24 reaction kit*, combine

0.7 µl NEBNext ARTIC Human Primer Mix 2 with

42 µl NEBNext VarSkip Short SARS-CoV-2 Primer Mix 2 in a new tube, vortex and spin down reagents. If using *96 reaction kit*, combine **2.8 µl NEBNext ARTIC Human Primer Mix 2** with

168 µl NEBNext VarSkip Short SARS-CoV-2 Primer Mix 2 in a new tube, vortex and spin down reagents.

Use **1.75 µl combined mix** for each Pool Set B reaction.

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

* If using NEBNext Human Primer Mix 2, add 1.75 µl of the combined NEBNext VarSkip Short SARS-CoV-2 Primer Mix 2 and NEBNext ARTIC Human Primer Mix 2.

5

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

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6

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

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

When cDNA amplification cleanup steps are skipped, the cDNA amplicon pool must be diluted before to proceeding to library preparation. Description of sample dilution is detailed below.

Dilution of cDNA Amplicons

7 Transfer 1.3 µl of the pooled cDNA amplicons to a fresh tube.

8 Add 11.7 µl of 0.1X TE for a final volume of 13 µl.

9

The pooled cDNA amplicons may be run on a TapeStation® to confirm 560 bp size of amplicons without cleaning up. Cleanup of cDNA amplicon pool is not needed for assessing on a TapeStation. To run on a TapeStation, dilute an aliquot of the pooled amplicons 10-fold with 0.1X TE Buffer and run 2 µl on a DNA High Sensitivity ScreenTape. (See Figure 9 below for example of amplicon size profile on a Tapestation)

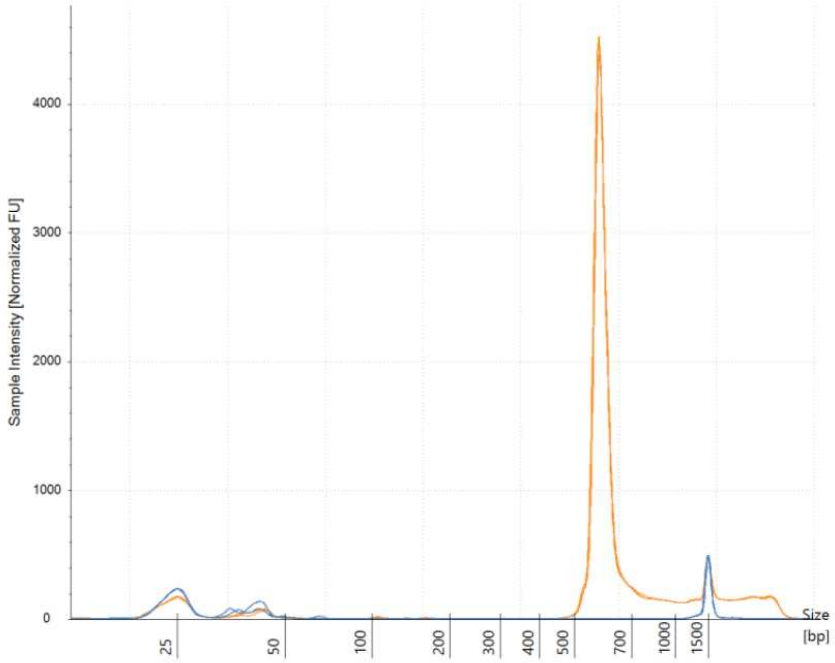

Figure 9: VarSkip Short SARS-CoV-2 cDNA amplicons generated from 1000 total viral copies. 1/10 diluted cDNA amplicons without bead cleanup run on a TapeStation.

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Fragmentation/End Prep

10

A	B	C
FRAGMENTATION SIZE RANGE	INCUBATION @ 37°C	OPTIMIZATION
80 bp–300 bp	25 min	20-30 min

Fragmentation occurs during a 37°C incubation step. A fragmentation time of 25 minutes should be sufficient for most samples to generate mainly ~120 bp fragments, however, the time may need to be optimized.

11

Ensure that the Ultra II FS Reaction Buffer is completely thawed. If a precipitate is seen in the buffer, pipette up and down several times to break it up, and quickly vortex to mix. Place **On ice** until use.

There are short videos on NEB.com about handling the FS reagents:
Preparing FS Reagents: https://www.neb.com/tools-and-resources/video-library/quick-tips---preparing-the-nebnext-ultra-ii-fs-dna-reaction-buffer-and-enzyme-mix
Making FS master mix: https://www.neb.com/tools-and-resources/video-library/quick-tips---preparing-nebnext-ultra-ii-fs-dna-reaction-buffer-and-enzyme-mix-master-mix

12

Vortex the Ultra II FS Enzyme Mix 5-8 seconds prior to use and place **On ice** .

It is important to vortex the enzyme mix prior to use for optimal performance

13

Add the following components to a 0.2 ml thin wall PCR tube **On ice** :

A	B
COMPONENT	VOLUME PER ONE LIBRARY
ARTIC SARS-CoV-2 cDNA (Step 8)	13 µl
(yellow) NEBNext Ultra II FS Reaction Buffer	3.5 µl
(yellow) NEBNext Ultra II FS Enzyme Mix	1 µl
Total Volume	17.5 µl

14 5s

Vortex the reaction for **00:00:05** and briefly spin in a microcentrifuge.

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15

In a thermocycler* run the following program:

A	B
TEMP	TIME
37°C	25 minutes
65°C	30 minutes
4°C	∞

*Set heated lid to 75°C

If necessary, samples can be stored at -20 °C ; however, a slight loss in yield (~20%) may be observed. We recommend continuing with adaptor ligation before stopping.

Adaptor Ligation

16

Add the following components directly to the FS Reaction Mixture:

A	B
COMPONENT	VOLUME
FS Reaction Mixture (Previous Step)	17.5 µl
(red) NEBNext Adaptor for Illumina**	1.25 µl
(red) NEBNext Ultra II Ligation Master Mix*	15 µl
Total Volume	33.75 µl

* Mix the Ultra II Ligation Master Mix by pipetting up and down several times prior to adding to the reaction.
** The NEBNext adaptor is provided in NEBNext Oligo kits. NEB has several oligo options which are supplied separately from the library prep kit. Please see www.neb.com/oligos for additional information

Do not premix adaptor with the Ligation Master Mix

17

Set a 100 µl or 200 µl pipette to 25 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.

Caution: The NEBNext Ultra II Ligation Master Mix is very viscous. Care should be taken to ensure adequate mixing of the ligation reaction, as incomplete mixing will result in reduced ligation efficiency. The presence of a small amount of bubbles will not interfere with performance.

18

15m

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Incubate at **20 °C** for **00:15:00** in a thermocycler with the heated lid off.

19 

Add **1.5 µl (red or blue) USER® Enzyme** to the ligation mixture from the previous step.

Steps 19 and 20 are only required for use with NEBNext Adaptors. USER enzyme can be found in the NEBNext Multiplex Oligos (www.neb.com/oligos).

20  15m

Mix well and incubate at **37 °C** for **00:15:00** with the heated lid set to ≥ 47°C.

Samples can be stored Overnight at -20 °C .

Note: Only a portion of the ligation reaction (7.5 µl) will move forward to PCR enrichment.

PCR Enrichment of Adaptor-ligated DNA

21

Follow step-case A. if you are using the following oligos (10 µM primer):
Use option A for any NEBNext Oligo kit where index primers are supplied in tubes. These kits have the forward and reverse primers supplied in separate tubes at 10 µM.

Follow step-case B. if you are using the following oligos (10 µM primers):
Use Option B for any NEBNext Oligo kit where index primers are supplied in a 96-well plate format. These kits have the forward and reverse (i7 and i5) primers combined. Primers are supplied at 10 µM.
Step 21 includes a Step case.
A. Forward and Reverse Primers Separate
B. Forward and Reverse Primers Combined

step case

A. Forward and Reverse Primers Separate

Index primers are supplied in tubes. These kits have the forward and reverse primers supplied in separate tubes.

22 Add the following components to a sterile strip tube:

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A	B
COMPONENT	VOLUME
Adaptor Ligated DNA Fragments (Step 20)	7.5 µl
(blue) NEBNext Library PCR Master Mix	12.5 µl
Index Primer/i7 Primer*,**	2.5 µl
Universal PCR Primer/i5 Primer*,**	2.5 µl
Total Volume	25 µl

* NEBNext Oligos must be purchased separately from the library prep kit. Refer to the corresponding NEBNext Oligo kit manual for determining valid barcode combinations.
** Use only one i7/primer/index primer per sample. Use only one i5 primer (or the universal primer for single index kits) per sample.

23 

Set a 100 µl pipette to 20 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.

24 

Place the tube on a thermocycler and perform PCR amplification using the following PCR cycling conditions:

A	B	C	D
CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	5*
Annealing/Extension	65°C	75 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

Set heated lid to 105°C.
* The number of PCR cycles recommended should be viewed as a starting point and may need to be optimized for particular sample types.

25 Proceed to Cleanup of PCR Amplification in the next step.

Cleanup of PCR Reaction 7m

26

The NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step. These volumes may not work properly for a cleanup at a different step in the workflow. For cleanups of samples contained in different buffer conditions, the volumes may need to be experimentally determined.

27 Vortex NEBNext Sample Purification Beads to resuspend.
Step 27 includes a Step case.
Individual Rxn
Pooled Rxn

step case

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Individual Rxn

Follow Section Option A if you will normalize individual final libraries prior to pooling for sequencing. Option A is recommended for samples with a large range of SARS-CoV-2 viral genome copies input into the cDNA synthesis reaction (Step 1), such as a set of samples with inputs **outside** of the 100–100,000 genome copies range.

28 

Add **17.5 µl (0.7 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.

29 

5m

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

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

31 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.

32 

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.

33 

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.

34 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.

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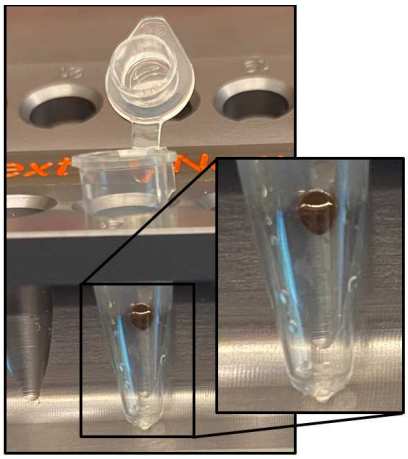
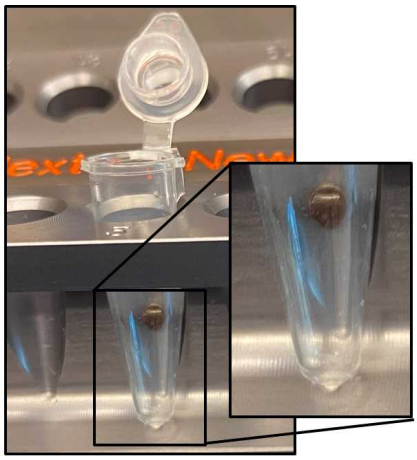


After ethanol is removed the beads will be shiny and droplets of ethanol will be on the inside of the tube



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

35

Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding **17 µl** **0.1X TE** .

36 2m

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

37

Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer **15 µl** to a new PCR tube and store at **-20 °C** .

38 Assess the library size distribution with Agilent Bioanalyzer or TapeStation high sensitivity DNA reagents. The sample may need to be diluted before loading. A peak size of 200-400 bp is expected, based on a 25-minute fragmentation time. (Figure 38)

If excess adaptor dimer peak is observed at 150–180 bp, a second 0.7X bead cleanup can be performed. The second 0.7X cleanup will result in slightly larger libraries which will not affect sequencing results.

Citation: New England Biolabs (09/20/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 FS Library Prep Kit (IlluminaÃÂ®) E7658ÃÂ Express Protocol with One Clean-up StepÃÂ . https://dx.doi.org/10.17504/protocols.io.bx9apr2e

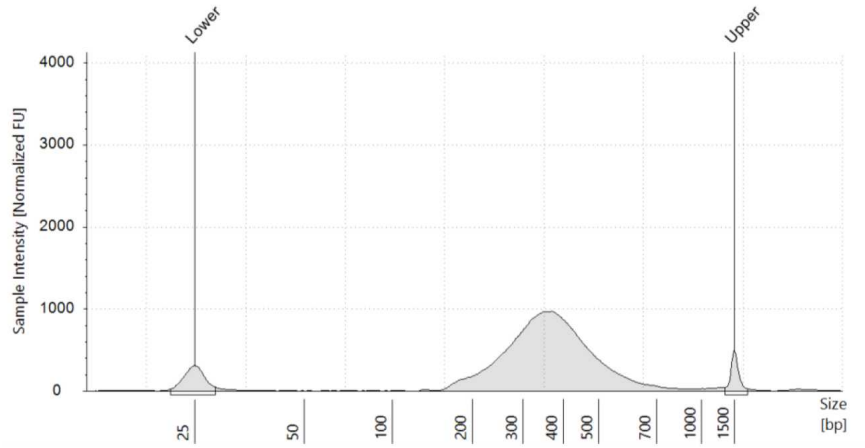


Figure 38: Example of final library pool size distributions on a TapeStation . VarSkip Short SARS-CoV-2 libraries were generated from 1,000 viral copies. Normalized library pool after 0.7X bead cleanup of individual libraries, pooling, and 0.7X bead ration cleanup of normalized pool.

Citation: New England Biolabs (09/20/2021). NEBNextÃÂ® Varskip Short ARTIC SARS-CoV-2 FS Library Prep Kit (IlluminaÃÂ®) E7658ÃÂ Express Protocol with One Clean-up StepÃÂ . https://dx.doi.org/10.17504/protocols.io.bx9apr2e