Version 2

Mar 04, 2021

NEBNext® ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®) E7658 V.2

New England Biolabs[1]

[1]New England Biolabs

1 Works for me dx.doi.org/10.17504/protocols.io.bsy5nfy6

New England Biolabs (NEB) Coronavirus Method Development Community

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SUBMIT TO PLOS ONE

ABSTRACT

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

EXTERNAL LINK

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

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

PROTOCOL CITATION

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GUIDELINES

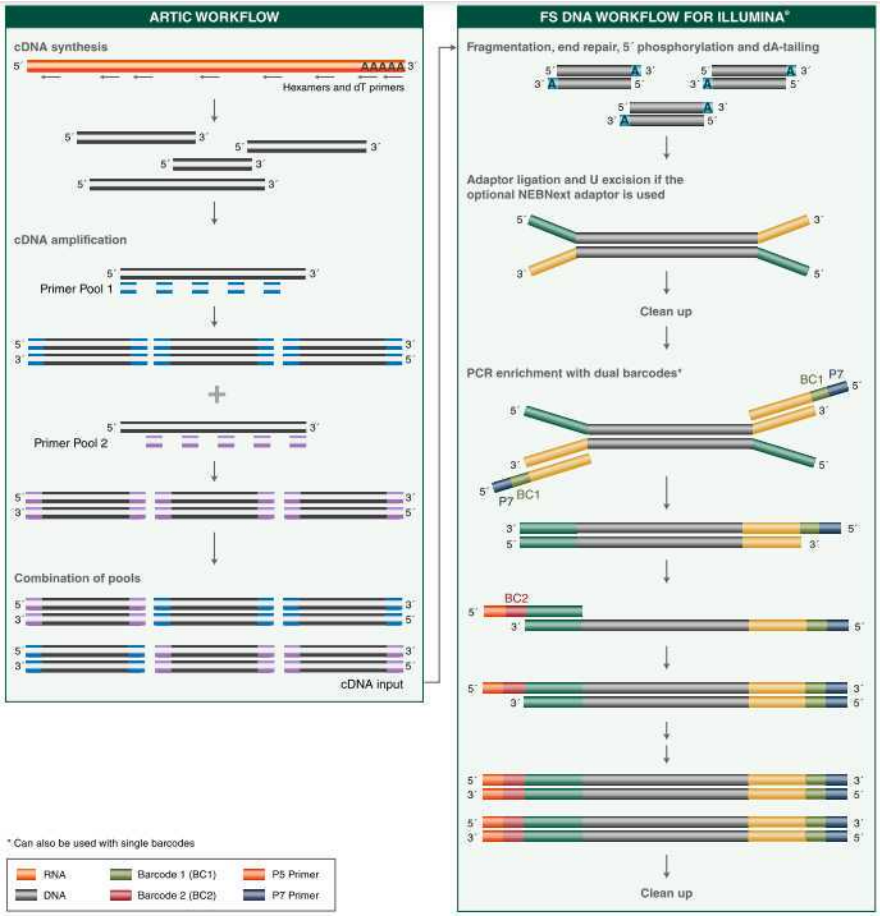
Overview

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.



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 (5X)
(lilac) Q5® Hot Start High-Fidelity 2X Master Mix
(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

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(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

- NEBNext Singleplex or Multiplex Oligos for Illumina – www.neb.com/oligos
- 80% Ethanol (freshly prepared)
- Nuclease-free Water
- DNA LoBind Tubes (Eppendorf® #022431021)
- Magnetic rack/stand (NEB #S1515, Alpaqua®, cat. #A001322 or equivalent)
- Thermocycler
- 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 (5X)	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
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

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A	B	C
NEB #	**PRODUCT**	**VOLUME**
E7651AA	LunaScript RT SuperMix (5X)	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
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

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

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: 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, however, results may vary depending on the quality of the 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

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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:

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.

3 Proceed to cDNA Amplification (Next Step).

cDNA Amplification

4

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.

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

5

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

For Pool Set A:

If using the ARTIC Human Primer Mix and a *24 reaction kit*, combine **0.7 µl ARTIC Human Primer Mix 1** with **42 µl ARTIC 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 ARTIC Primer Mix 1** with **168 µl ARTIC 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
ARTIC SARS-CoV-2 Primer Mix 1 *	1.75 µl
Total Volume	12.5 µl

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

For Pool Set B:

If using the ARTIC Human Primer Mix and a *24 reaction kit*, combine **0.7 µl ARTIC Human Primer Mix 2** with **42 µl ARTIC SARS-CoV-2 Primer Mix 2** in a new tube, vortex and spin down reagents. If using *96 reaction kit*, combine **2.8 µl ARTIC Human Primer Mix 2** with **168 µl ARTIC 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
ARTIC SARS-CoV-2 Primer Mix 2 *	1.75 µl
Total Volume	12.5 µl

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

6

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

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*Set heated lid to 105°C

7

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.

8 Proceed to cDNA Amplicon cleanup (Next Step).

Cleanup of cDNA Amplicons

9

The volume of NEBNext Sample Purification Beads provided here are for use with the sample composition at this step (25 µl; Step 7). 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.

10 Vortex NEBNext Sample Purification Beads to resuspend.

11

Add **20 µl (0.8X) resuspended beads** to the combined 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.

12 5m

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

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

14 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 (03/04/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 FS Library Prep Kit (IlluminaÃÂ®) E7658. https://dx.doi.org/10.17504/protocols.io.bsy5nfy6

15 30s

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.

16 30s

Repeat 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.

17 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.

18

Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding **16 µl 0.1X TE** . If not assessing amplicons (Step 21) elute DNA in **15 µl 0.1X TE** .

19 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.

20

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 not assessing amplicons (Step 21) transfer **13 µl** to a new PCR tube.

21

We recommend assessing cDNA amplicon (from Step 20) concentrations with a Qubit fluorometer.

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Amplicons may also be run on a Bioanalyzer or TapeStation® to confirm 400 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 21 below for example of amplicon size profile on a Bioanalyzer).

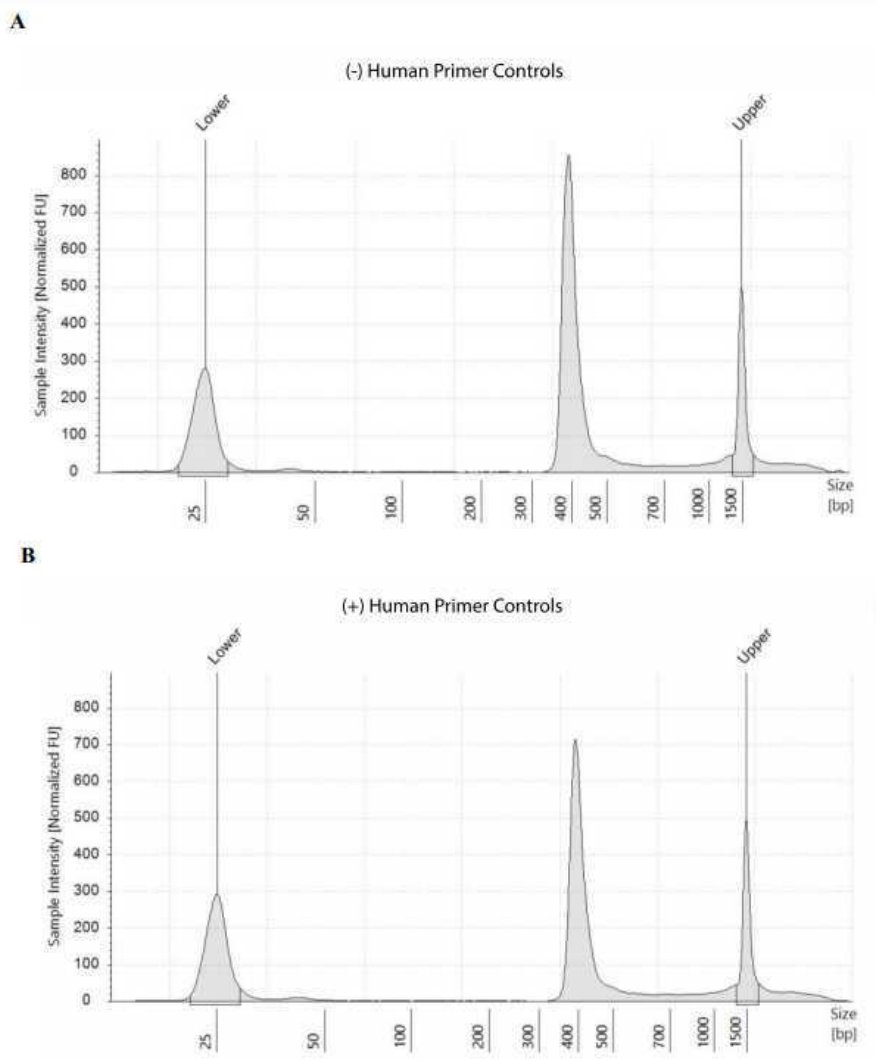

Figure 21: ARTIC SARS-CoV-2 cDNA amplicons generated from 1000 total viral copies in the absence (A) or presence (B) of the human primer controls.

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

22 Proceed to Library Prep in the Next Step.

Fragmentation/End Prep

Citation: New England Biolabs (03/04/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 FS Library Prep Kit (IlluminaÃÂ®) E7658. https://dx.doi.org/10.17504/protocols.io.bsy5nfy6

23

A	B	C
FRAGMENTATION SIZE RANGE	INCUBATION @ 37°C	OPTIMIZATION
80 bp–250 bp	30 min	30–40 min

Fragmentation occurs during a 37°C incubation step. A fragmentation time of 30 minutes should be sufficient for most samples to generate mainly 100 bp fragments, however, the time may need to be optimized. For high input samples (1 µg), fragmentation time may need to be increased.

24

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

25

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

26

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 20)	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

27

5s

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

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28

In a thermocycler* run the following program:

A	B
TEMP	TIME
37°C	30 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

29

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

30

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.

31

15m

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

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32 

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

Steps 32 and 33 are only required for use with NEBNext Adaptors. USER enzyme can be found in the NEBNext Multiplex Oligos (www.neb.com/oligos).

33 

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 .

Cleanup of Adaptor-ligated cDNA

34 **The following section is for cleanup of the ligation reaction.**

The volumes of NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step (35.25 µl; Step 33). 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

35 Vortex the NEBNext Sample Purification Beads to resuspend.

36 

Add **28 µl (0.8X) resuspended beads** to the Adaptor Ligation 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.

37 

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

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

Citation: New England Biolabs (03/04/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 FS Library Prep Kit (IlluminaÃÂ®) E7658. https://dx.doi.org/10.17504/protocols.io.bsy5nfy6

39 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.

40 

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.

41 

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.

42 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.

43 

Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding **10 µl 0.1X TE** .

44 

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.

45 

Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer **7.5 µl** to a new PCR tube.

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Samples can be stored at 🌡 **-20 °C .**

46 Proceed to PCR Enrichment of Adaptor-ligated DNA in the next step.

PCR Enrichment of Adaptor-ligated DNA

47

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.

Follow step-case B. if you are using the following oligos (10 µM primer):
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.
Step 47 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.

48 Add the following components to a sterile strip tube:

A	B
COMPONENT	VOLUME
Adaptor Ligated DNA Fragments (Step 45)	7.5 µl
(blue) NEBNext Library PCR Master Mix	12.5 µl
Index Primer/i7 Primer*,**	2.5 µl
(blue) 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.

49

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.

50

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

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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.

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

Cleanup of PCR Reaction 7m

52

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.

53 Vortex NEBNext Sample Purification Beads to resuspend.

54 

Add **22.5 µl (0.9X) 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.

55  5m

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

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

57 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 (03/04/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 FS Library Prep Kit (IlluminaÃÂ®) E7658. https://dx.doi.org/10.17504/protocols.io.bsy5nfy6

58

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.

59

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.

60 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.

61

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

62 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.

63

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** .

64 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 200–250 bp is expected, based on a 30-minute fragmentation time. (Figure 64)

Citation: New England Biolabs (03/04/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 FS Library Prep Kit (IlluminaÃÂ®) E7658. https://dx.doi.org/10.17504/protocols.io.bsy5nfy6

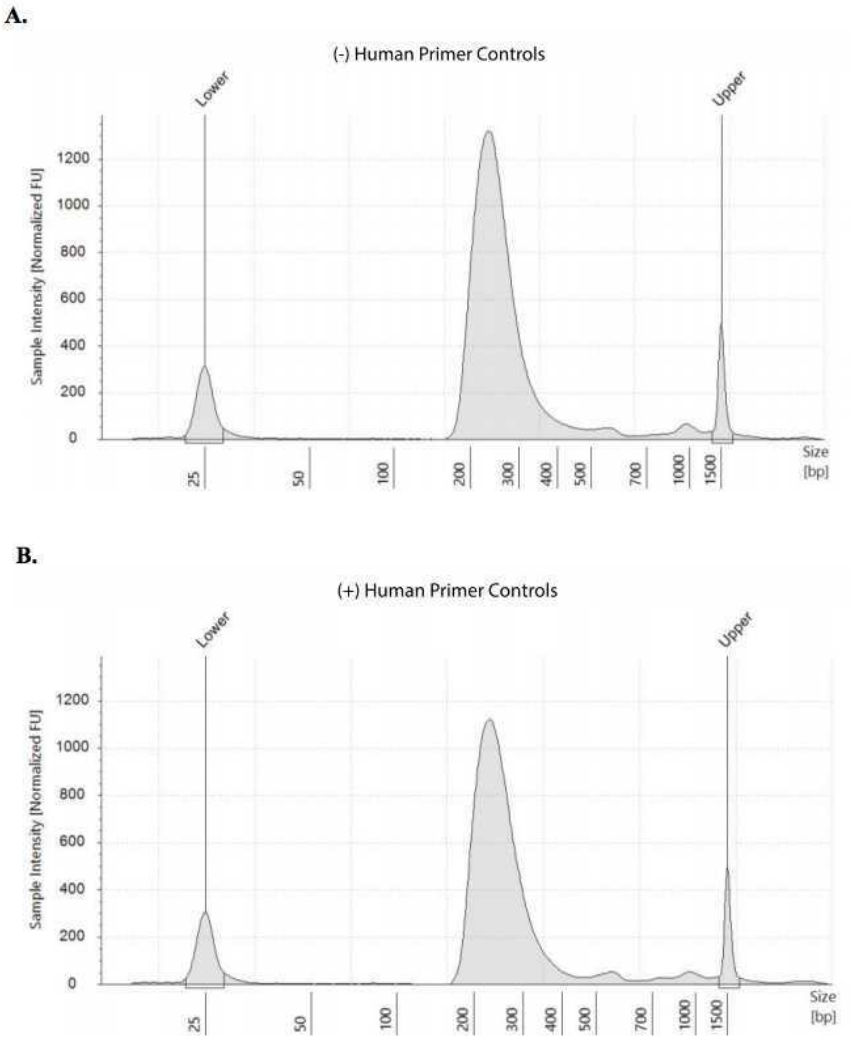

Figure 64. : Example of final library size distributions on a TapeStation. ARTIC SARS-CoV-2 libraries were generated from 1000 viral copies in the absence (A) or presence (B) of the human primer controls.

Citation: New England Biolabs (03/04/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 FS Library Prep Kit (IlluminaÃÂ®) E7658. https://dx.doi.org/10.17504/protocols.io.bsy5nfy6