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# NEBNext® ARTIC SARS-CoV-2 Library Prep Kit (Illumina®) (NEB #E7650S/L)

Isabel Gautreau[1]

[1]New England Biolabs

1 *Works for me* dx.doi.org/10.17504/protocols.io.br7dm9i6

New England Biolabs (NEB) Coronavirus Method Development Community

Isabel Gautreau
New England Biolabs

SUBMIT TO PLOS ONE

ABSTRACT

This protocol is about the NEBNext® ARTIC SARS-CoV-2 Library Prep Kit (Illumina®).

EXTERNAL LINK

https://www.neb.com/-/media/nebus/files/manuals/manuale7650.pdf?rev=f37be6e42b4f486f888f204b83907d3f&hash=69F40067B46B925A7D45F5A6D418095F

ATTACHMENTS

NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina) E7650.pdf

DOI

dx.doi.org/10.17504/protocols.io.br7dm9i6

EXTERNAL LINK

https://www.neb.com/-/media/nebus/files/manuals/manuale7650.pdf?rev=f37be6e42b4f486f888f204b83907d3f&hash=69F40067B46B925A7D45F5A6D418095F

PROTOCOL CITATION

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KEYWORDS

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Feb 08, 2021 Julia Rossmanith protocols.io

Feb 25, 2021 Isabel Gautreau New England Biolabs

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GUIDELINES

**Overview**

The NEBNext SARS-CoV-2 Library Prep Kit (Illumina) contains the enzymes, buffers and oligos required to convert a broad range of total RNA input amounts into targeted, high quality 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 an indexed library on the 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.

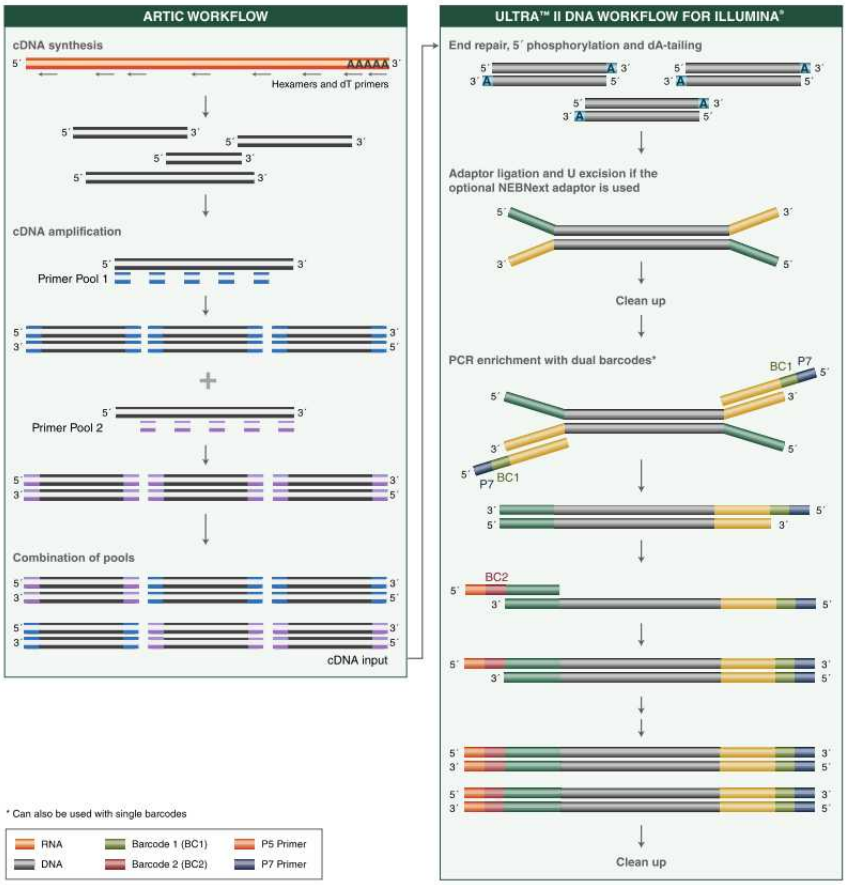


**Figure 1. Workflow demonstrating the use of NEBNext SARS-CoV-2 Library Prep Kit for Illumina**

**NEBNext ARTIC Human Primers**



**Citation:** Isabel Gautreau (02/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Library Prep Kit (IlluminaÃÂ®)ÃÂ (NEB #E7650S/L). https://dx.doi.org/10.17504/protocols.io.br7dm9i6

| A | B | C | D |
|---|---|---|---|
| PRIMER MIX | GENE | POSITION | PRIMERS |
| NEBNext ARTIC Human Primer Mix 1 | EDF1 | 113 bp – 501 bp | GGCCAAATCCAAGCAGGCTA<br>GCTTTGTGTTCATTTCGCCCT |
| NEBNext ARTIC Human Primer Mix 2 | NEDD8 | 110 bp – 489 bp | AAAGTGAAGACGCTGACCGG<br>GGGATCCTCACAGTCTCCCA |

**NEBNext ARTIC SARS-CoV-2 Primer Mix 1 and 2**
NEBNext ARTIC SARS-CoV-2 Mix 1 and 2 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

MATERIALS TEXT

**Kit:**
NEBNext® ARTIC SARS-CoV-2 Library Prep Kit (Illumina®)
NEB #E7650S/L (24/96 reactions)

**The Library Kit Includes:**
*The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7650S) and 96 reactions (NEB #E7650L). Colored bullets represent the color of the cap of the tube containing the reagent.*

**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
(green) NEBNext Ultra II End Prep Enzyme Mix
(green) NEBNext Ultra II End Prep 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 #E7650S Table of Components



**Citation:** Isabel Gautreau (02/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Library Prep Kit (IlluminaÃÂ®)ÃÂ (NEB #E7650S/L). https://dx.doi.org/10.17504/protocols.io.br7dm9i6

| A | B | C |
|---|---|---|
| NEB # | PRODUCT | VOLUME |
| E7651A | LunaScript RT SuperMix | 0.048 ml |
| E7652A | Q5 Hot Start High-Fidelity 2X Master Mix | 0.30 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 |
| E7653A | NEBNext Ultra II End Prep Enzyme Mix | 0.046 ml |
| E7654A | NEBNext Ultra II End Prep 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 Buffer | 1.3 ml |
| E7667A | Nuclease-free Water | 1.5 ml |
| E7104S | NEBNext Sample Purification Beads | 2.1 ml |

NEB #E7650L 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 | 1.20 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 |
| E7653AA | NEBNext Ultra II End Prep Enzyme Mix | 0.144 ml |
| E7654AA | NEBNext Ultra II End Prep Reaction Buffer | 0.336 ml |
| E7655AA | NEBNext Ultra II Ligation Master Mix | 0.72 ml |
| E7656AA | NEBNext Library PCR Master Mix | 1.20 ml |
| E7657AA | 0.1X TE Buffer | 5.20 ml |
| E7667AA | Nuclease-free Water | 1.50 ml |
| E7104L | NEBNext Sample Purification Beads | 8 ml |

**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<br>GTGTTCATTTCGCCCTAGGC |
| NEBNext ARTIC Human Primer Mix 2 | NEDD8 | 110 bp – 489 bp | AAAGTGAAGACGCTGACCGG<br>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 Primer Mix 1 and 2**

NEBNext ARTIC SARS-CoV-2 Mix 1 and 2 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 the Safety Data Sheets (SDS) for health and environmental hazards.

BEFORE STARTING



**Citation:** Isabel Gautreau (02/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Library Prep Kit (IlluminaÃÂ®)ÃÂ (NEB #E7650S/L). https://dx.doi.org/10.17504/protocols.io.br7dm9i6

**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 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 targeted cDNA amplification.



**Citation:** Isabel Gautreau (02/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Library Prep Kit (IlluminaÃÂ®)ÃÂ (NEB #E7650S/L). https://dx.doi.org/10.17504/protocols.io.br7dm9i6

## Targeted 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.**

**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** , 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** , vortex and spin down reagents. Use **1.75 µl of the 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** , vortex and spin down reagents. If using a *96 reaction kit*, combine **2.8 µl ARTIC Human Primer Mix 2** with **168 µl ARTIC SARS-CoV-2 Primer Mix 2** . Use **1.75 µl of the 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 nCoV-2019 Primer Pool 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.



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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 |

*Set heated lid to 105°C

7

Combine the Pool A and Pool B PCR reactions.

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

8 Proceed to cDNA amplicon cleanup.

## 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 the 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** .



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13 

Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

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**.

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 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.**

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 **28 µl 0.1X TE** . If not assessing cDNA (Step 21) elute DNA in **27 µl 0.1X TE** .

19 

Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes 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



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20

Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer **26 µl** to a new PCR tube. If not assessing cDNA (Step 21) transfer **25 µl** to a new PCR tube.

21

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

**Amplicons may also be run on a Bioanalyzer or TapeStation® to confirm 400 bp size of amplicons. To run on a Bioanalyzer, 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).**

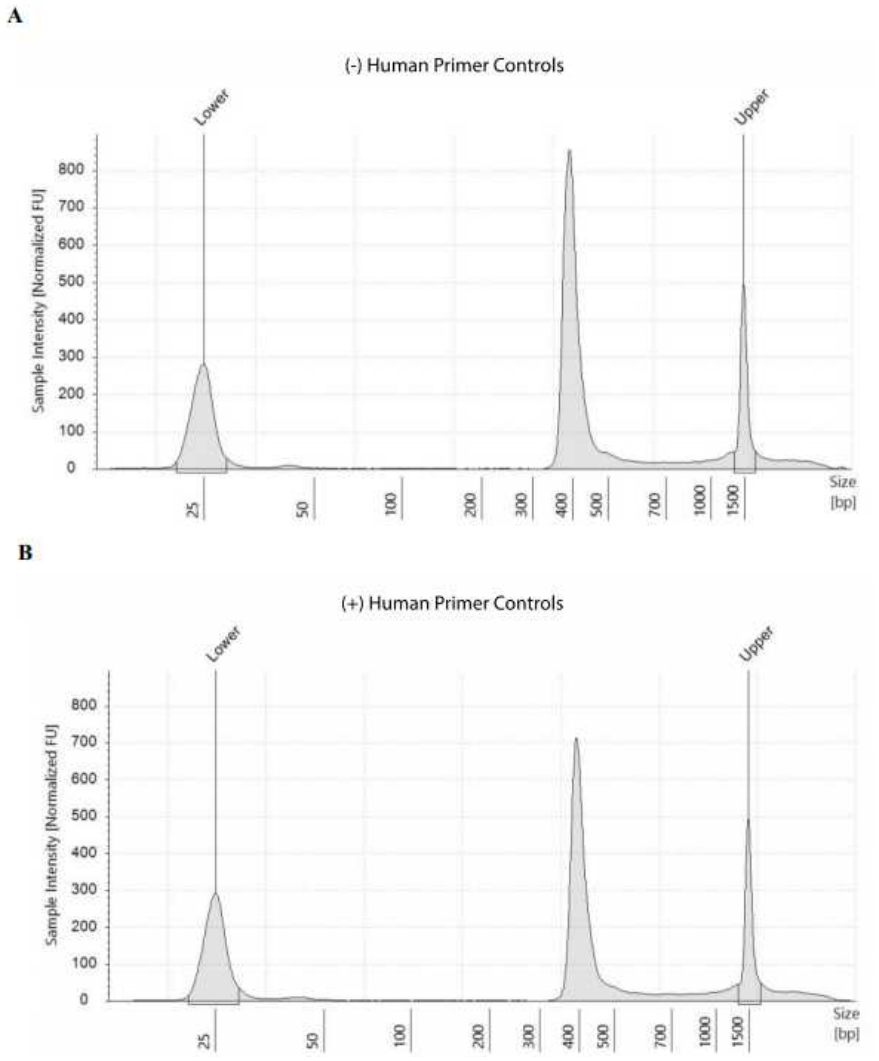


**Figure 21: Example of cDNA amplicons generated from 1000 genome copies of SARS CoV-2 in the absence (A) and presence (B) of human primer controls.**



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

22 Proceed to Library Prep in the next section.

## NEBNext End Prep

23 

Add the following components to a sterile nuclease-free tube:

| A | B |
|---|---|
| COMPONENT | VOLUME |
| (green) NEBNext Ultra II End Prep Enzyme Mix | 1.5 µl |
| (green) NEBNext Ultra II End Prep Reaction Buffer | 3.5 µl |
| Targeted cDNA Amplicons (Step 20) | 25 µl |
| *Total Volume* | 30 µl |

24 

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.

**It is important to mix well. The presence of a small amount of bubbles will not interfere with performance**

25 

Place in a thermocycler and run the following program:

| A | B |
|---|---|
| TEMP | TIME |
| 20°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.**



**Citation:** Isabel Gautreau (02/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Library Prep Kit (IlluminaÃÂ®)ÃÂ (NEB #E7650S/L). https://dx.doi.org/10.17504/protocols.io.br7dm9i6

## Adaptor Ligation

26

Add the following components directly to the End Prep Reaction Mixture:

| A | B |
|---|---|
| COMPONENT | VOLUME |
| End Prep Reaction Mixture (previous step) | 30 µl |
| (red) NEBNext Adaptor for Illumina** | 1.25 µl |
| (red) NEBNext Ultra II Ligation Master Mix* | 15 µl |
| *Total Volume* | 46.25 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.**

27

Set a 100 µl or 200 µl pipette to 40 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.**

28

15m

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

29

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

**Steps 29 and 30 are only required for use with NEBNext Adaptors. USER enzyme can be found in the NEBNext Multiplex Oligos (www.neb.com/oligos).**

30

15m

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



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

## Cleanup of Adaptor-ligated DNA

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

**The volume of NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step (47.75 µl; Step 30). 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.**

32 Vortex the NEBNext Sample Purification Beads to resuspend.

33 

Add **43 µl (0.9X) 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.

34 

5m

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

35 Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

36 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 beads**.

37 

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.



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38 30s

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.**

39 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 target. 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.**

40

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

41 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.

42 5m

Place the tube/plate on the magnetic stand. After **00:05:00** (or when the solution is clear), transfer **7.5 µl** to a new PCR tube.

**Samples can be stored at -20 °C .**

43 Proceed to PCR Enrichment of Adaptor-ligated DNA.

PCR Enrichment of Adaptor-ligated DNA

44

**Check and verify that the concentration of your oligos is 10 Micromolar (µM) .**



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**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 44 includes a Step case.
**Tubes**
**96-well plate**

step case

## Tubes

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

45 

Add the following components to a sterile strip tube:

| A | B |
|---|---|
| COMPONENT | VOLUME |
| Adaptor Ligated DNA Fragments (Step 42) | 7.5 µl |
| (blue) NEBNext Library PCR Master Mix | 12.5 µl |
| (blue) Universal PCR Primer/i5 Primer *,** | 2.5 µl |
| (blue) Index (X) Primer/i7 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.

46 

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.

47 

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.

48 Proceed to Cleanup of PCR Amplification in the next section.



**Citation:** Isabel Gautreau (02/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Library Prep Kit (IlluminaÃÂ®)ÃÂ (NEB #E7650S/L). https://dx.doi.org/10.17504/protocols.io.br7dm9i6

## Cleanup of PCR Reaction 8m

49

**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.**

50 Vortex NEBNext Sample Purification Beads to resuspend.

51 

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.

52 

5m

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

53 Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

54 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.**

55 

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.

56 

30s

Repeat the previous step once for a total of two washes:



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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.**

57 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.**

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

59 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.

60

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** .

61

Check the size distribution on an Agilent Bioanalyzer or TapeStation. The sample may need to be diluted before loading. A peak size of ~520 bp is expected (Figure 61).

**Samples can be stored at -20 °C .**



**Citation:** Isabel Gautreau (02/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Library Prep Kit (IlluminaÃÂ®)ÃÂ (NEB #E7650S/L). https://dx.doi.org/10.17504/protocols.io.br7dm9i6

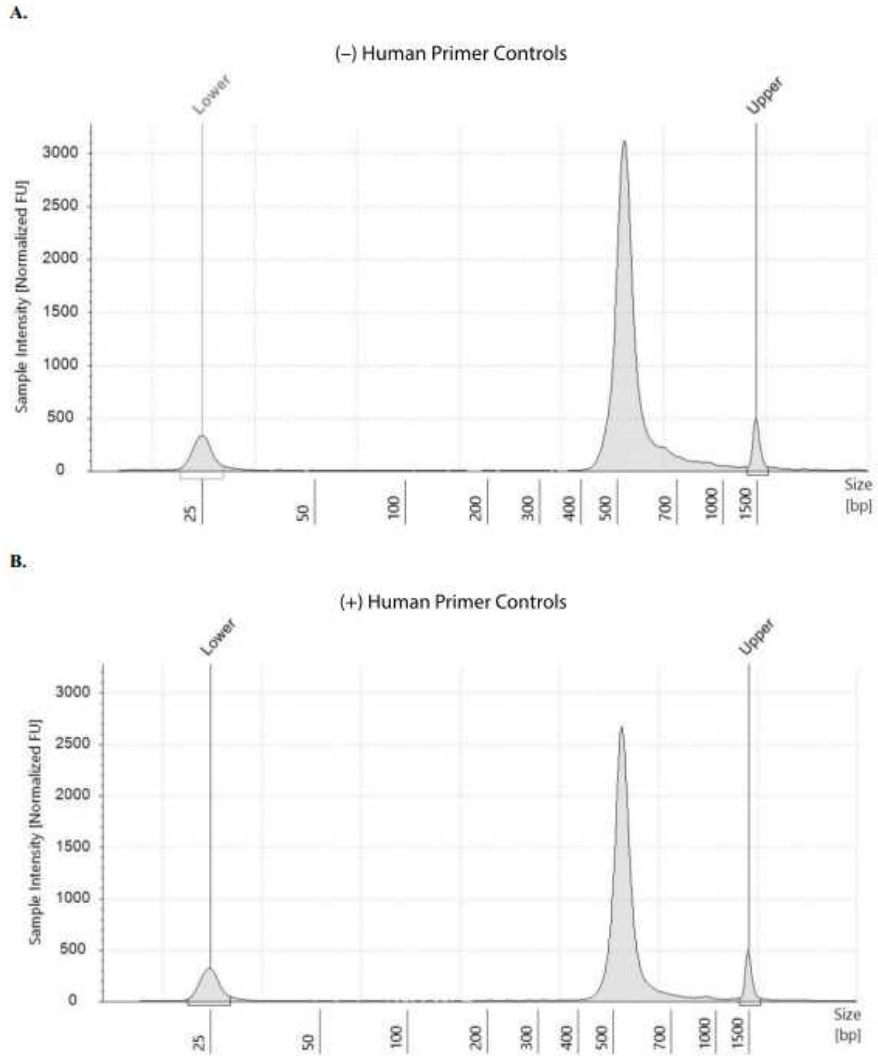


**Figure 61: Example of final library size distributions on a TapeStation. ARTIC SARS-CoV-2 libraries were generated from 1000 viral copies in the absence (A) or presence (B) of the human primer controls.**



**Citation:** Isabel Gautreau (02/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Library Prep Kit (IlluminaÃÂ®)ÃÂ (NEB #E7650S/L). https://dx.doi.org/10.17504/protocols.io.br7dm9i6