

NEBNext[®] ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina[®])

NEB #E7658S/L

24/96 reactions

Version 1.0_2/21

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The Library Kit Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7658S) and 96 reactions (NEB #E7658L). 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 Control Primer Pairs 1
- (lilac) NEBNext ARTIC Human Control Primer Pairs 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
- (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)

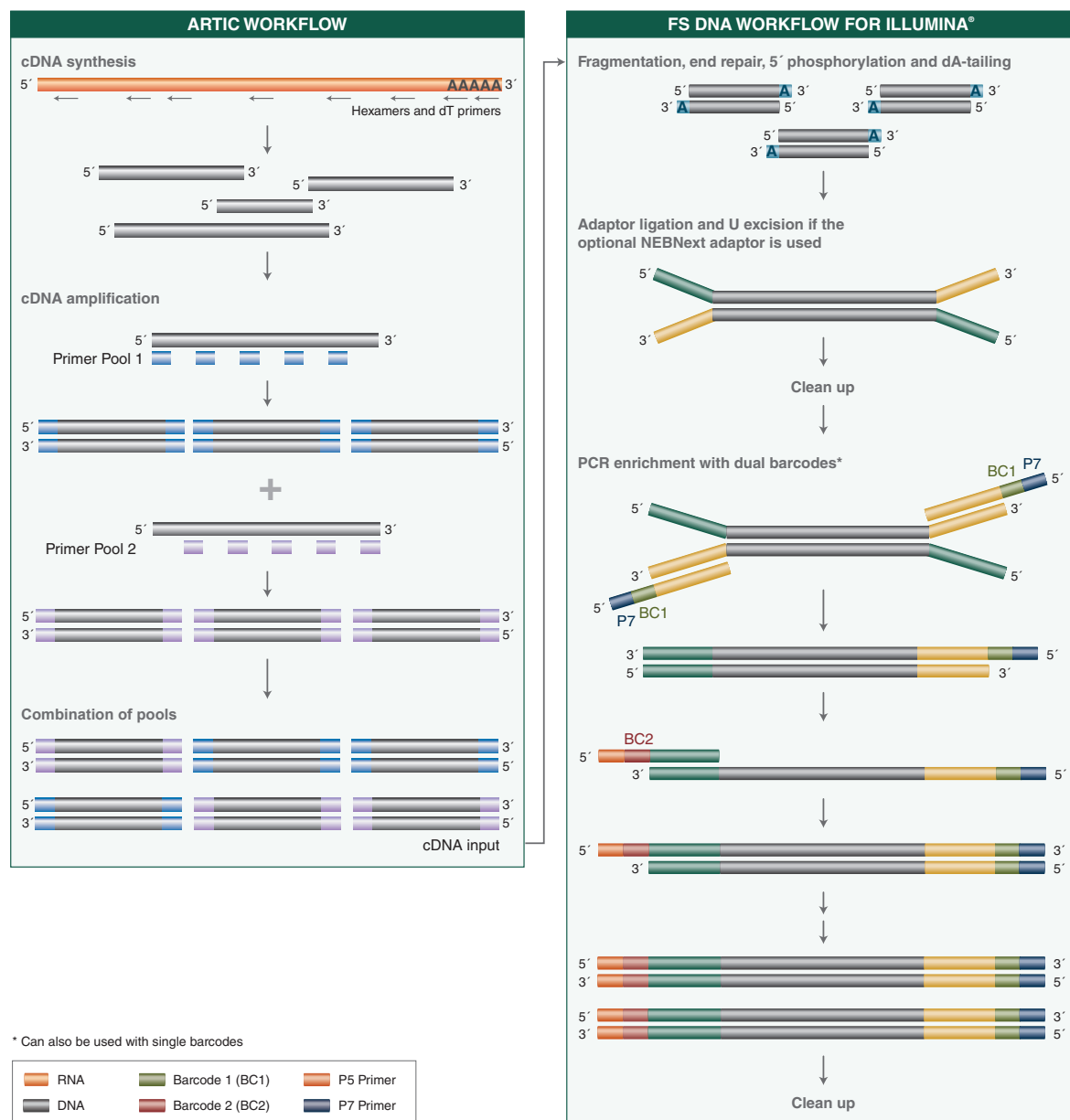
Overview

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 Control Primer Pairs 1) and NEDD8 (NEBNext ARTIC Human Control Primer Pairs 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.



Protocol

Symbols



This is a point where you can safely stop the protocol.



This caution sign signifies a step in the protocol that has two paths leading to the same end point but is dependent on a user variable, like the amount of input DNA.



Colored bullets indicate the cap color of the reagent to be added to a reaction.

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.

1. cDNA Synthesis

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.

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

| COMPONENT | VOLUME |
|----------------------------------|------------|
| RNA Sample | 8 μ l |
| ● (lilac) LunaScript RT SuperMix | 2 μ l |
| Total Volume | 10 μ l |

For no template controls, mix the following components:

| COMPONENT | VOLUME |
|----------------------------------|------------|
| ○ (white) Nuclease-free Water | 8 μ l |
| ● (lilac) LunaScript RT SuperMix | 2 μ l |
| Total Volume | 10 μ l |

- 1.2. Incubate reactions in a thermocycler with the following steps:

| 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.

- 1.3. Proceed to cDNA Amplification (Step 2).

2. cDNA Amplification

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

- 2.1. 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 Control Primer Pairs and a 24 reaction kit, combine 0.7 µl of NEBNext ARTIC Human Control Primer Pairs 1 with 42 µl of ARTIC SARS-CoV-2 Primer Mix 1, vortex and spin down reagents. If using a 96 reaction kit, combine 2.8 µl of the NEBNext ARTIC Human Control Primer Mix 1 with 168 µl of NEBNext 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.

| COMPONENT | VOLUME |
|--|---------|
| cDNA (Step 1.2) | 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 NEBNext ARTIC Human Control Primer Pairs 1, add 1.75 µl of the combined ARTIC SARS-CoV-2 Primer Mix 1 and NEBNext ARTIC Human Control Primer Pairs 1.

For Pool Set B:

If using the NEBNext ARTIC Human Control Primer Pairs and a 24 reaction kit, combine 0.7 µl of NEBNext ARTIC Human Control Primer Pairs 2 with 42 µl of ARTIC SARS-CoV-2 Primer Mix 2, vortex and spin down reagents. If using a 96 reaction kit, combine 2.8 µl of the NEBNext ARTIC Human Control Primer Pairs 2 with 168 µl of ARTIC SARS-CoV-2 Primer Mix 2. Use 1.75 µl of the combined mix for each Pool Set B reaction.

| COMPONENT | VOLUME |
|--|---------|
| cDNA (Step 1.2) | 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 NEBNext ARTIC Human Control Primer Pairs 2, add 1.75 µl of the combined ARTIC SARS-CoV-2 Primer Mix 2 and NEBNext ARTIC Human Control Primer Pairs 2.

- 2.2. Incubate reactions in a thermocycler with the following steps:

| 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

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



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

- 2.5. Proceed to cDNA Amplicon cleanup (Step 3).

3. Cleanup of cDNA Amplicons.



Note: The volume of NEBNext Sample Purification Beads provided here are for use with the sample composition at this step (25 µl; Step 2.3). 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.

- 3.1. Vortex NEBNext Sample Purification Beads to resuspend.
- 3.2. 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.
- 3.3. Incubate samples at room temperature for at least 5 minutes.
- 3.4. 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.
- 3.5. 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**).
- 3.6. Add 200 µl of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
- 3.7. Repeat Step 3.6. once for a total of two washes. 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.
- 3.8. 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.
- 3.9. 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 3.12.) elute DNA in 15 µl of 0.1X TE.
- 3.10. 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.
- 3.11. 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 3.12) transfer 13 µl to a new PCR tube.
- 3.12. We recommend assessing cDNA amplicon (from Step 3.11) concentrations with a Qubit fluorometer.

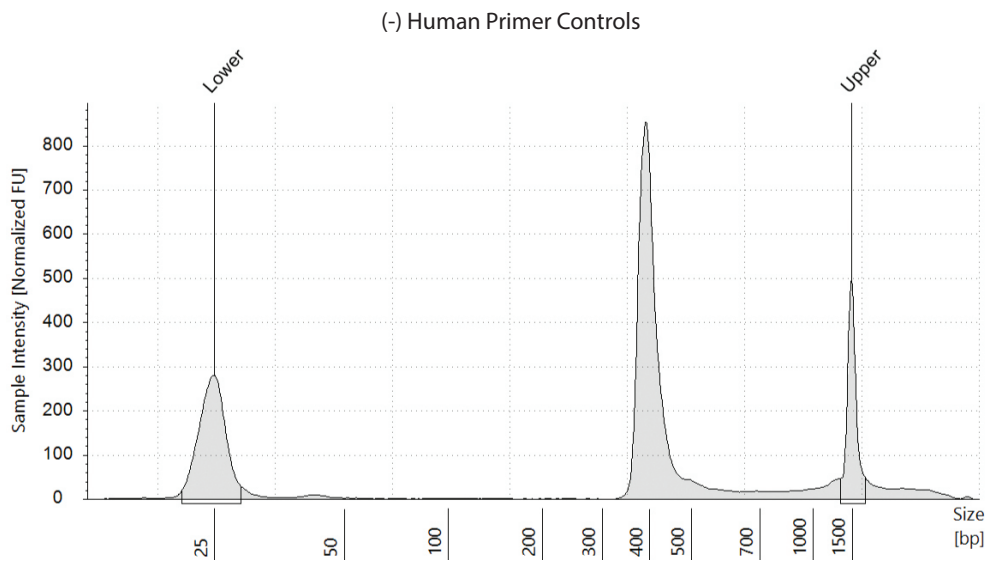
Note: Amplicons may also be run on a Bioanalyzer or TapeStation® to confirm 400 bp size of amplicons. To run on a TapeStation, dilute amplicon 10-fold with 0.1X TE Buffer and run 2 µl on a DNA High Sensitivity ScreenTape. (See Figure 3.13. below for example of amplicon size profile on a Bioanalyzer).



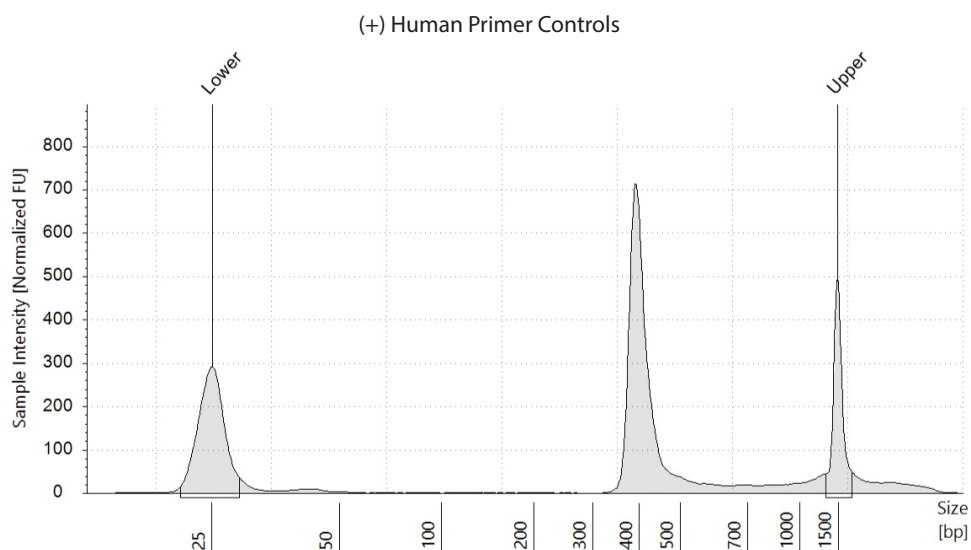
Samples can be stored at –20°C for up to a week.

Figure 3.13: ARTIC SARS-CoV-2 cDNA amplicons generated from 1000 total viral copies in the absence (A) or presence (B) of the human primer controls.

A.



B.



3.14. Proceed to Library Prep in Step 4.

4. Fragmentation/End Prep

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

Note: 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.

- 4.1. 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.
- 4.2. Vortex the Ultra II FS Enzyme Mix 5-8 seconds prior to use and place on ice.

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

- 4.3. Add the following components to a 0.2 ml thin wall PCR tube on ice:

| COMPONENT | VOLUME PER ONE LIBRARY |
|--|------------------------|
| ARTIC SARS-CoV-2 cDNA (Step 3.11) | 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 |

- 4.4. Vortex the reaction for 5 seconds and briefly spin in a microcentrifuge.

- 4.5. In a thermocycler, run the following program:

| 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.

5. Adaptor Ligation

- 5.1. Add the following components directly to the FS Reaction Mixture:

| COMPONENT | VOLUME |
|---|----------|
| FS Reaction Mixture (Step 4.5) | 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.

Note: Do not premix adaptor with the Ligation Master Mix.

- 5.2. 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).**

- 5.3. Incubate at 20°C for 15 minutes in a thermocycler with the heated lid off.

- 5.4. Add 1.5 µl of ● (red) USER[®] Enzyme to the ligation mixture from Step 5.3.

Note: Steps 5.4. and 5.5. are only required for use with NEBNext Adaptors. USER enzyme can be found in the NEBNext Multiplex Oligos (www.neb.com/oligos).

- 5.5. Mix well and incubate at 37°C for 15 minutes with the heated lid set to ≥ 47°C.



Samples can be stored overnight at –20°C.

6. Cleanup of Adaptor-ligated cDNA



The following section is for cleanup of the ligation reaction.

Note: 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 5.5). 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.

- 6.1. Vortex the NEBNext Sample Purification Beads to resuspend.
- 6.2. 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.
- 6.3. Incubate samples at room temperature for at least 5 minutes.
- 6.4. 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.
- 6.5. 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**).
- 6.6. Add 200 µl of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
- 6.7. Repeat Step 6.6 once for a total of two washes. 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.
- 6.8. 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.

- 6.9. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 10 µl of 0.1X TE.
- 6.10. 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.
- 6.11. 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.



Samples can be stored at -20°C .

- 6.12. Proceed to PCR Enrichment of Adaptor-ligated DNA in Step 7.

7. PCR Enrichment of Adaptor-ligated DNA



Note: Check and verify that the concentration of your oligos is 10 µM.



Follow Section 7.1.1A. 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 Section 7.1.1B. 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.

7.1. PCR Amplification

7.1.1. Add the following components to a sterile strip tube:

7.1.1A. Forward and Reverse Primers Separate

| COMPONENT | VOLUME |
|---|---------|
| Adaptor Ligated DNA Fragments (Step 6.11) | 7.5 µl |
| • (blue) NEBNext Library PCR Master Mix | 12.5 µl |
| • (blue) 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.

7.1.1B. Forward and Reverse Primers Combined

| COMPONENT | VOLUME |
|---|---------|
| Adaptor Ligated DNA Fragments (Step 6.11) | 7.5µl |
| • (blue) NEBNext Library PCR Master Mix | 12.5 µl |
| • (blue) Index/Universal Primer* | 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

7.1.2. 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.

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

| 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.

7.1.4. Proceed to Cleanup of PCR Amplification in Step 8.

8. Cleanup of PCR Reaction

Note: 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.

8.1. Vortex NEBNext Sample Purification Beads to resuspend.

8.2. 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.

8.3. Incubate samples on bench top for at least 5 minutes at room temperature.

8.4. 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.

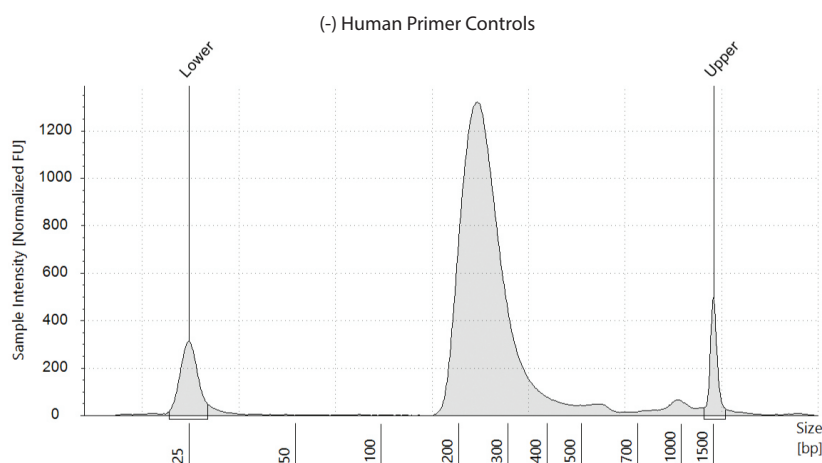
- 8.5. After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (**Caution: do not discard the beads**).
- 8.6. Add 200 μ l of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
- 8.7. Repeat Step 8.6. once for a total of two washes. 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.
- 8.8. 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.

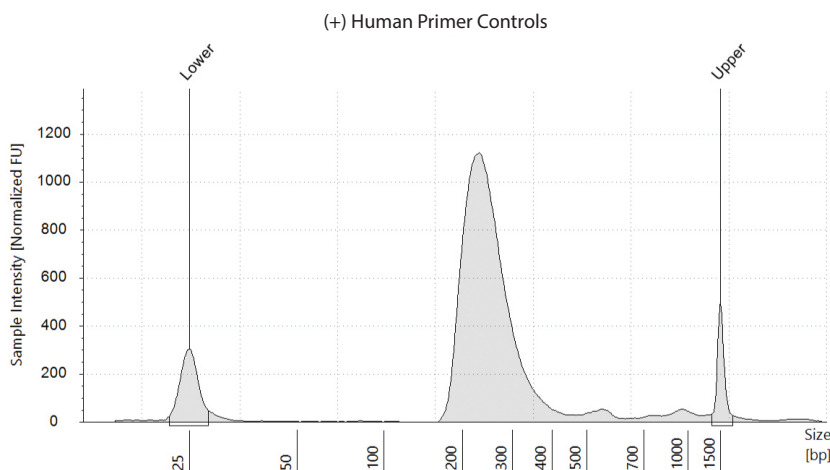
- 8.9. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 17 μ l of 0.1X TE.
- 8.10. Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.
- 8.11. Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 15 μ l to a new PCR tube and store at -20°C .
- 8.12. 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 sized of 200–250 bp is expected, based on a 30-minute fragmentation time (Figure 3).

Figure 8.12: 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.

A.



B.



Kit Components

NEB #E7658S Table of Components

| 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 Control Primer Pairs 1 | 7 µl |
| E7728A | NEBNext ARTIC Human Control Primer Pairs 2 | 7 µl |
| 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

| 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 |
| E7727A | NEBNext ARTIC Human Control Primer Pairs 1 | 7 µl |
| E7728A | NEBNext ARTIC Human Control Primer Pairs 2 | 7 µl |
| 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 |
| E7667A | Nuclease-free Water | 1.5 ml |
| E7659L | NEBNext Sample Purification Beads | 4 x 2.1 ml |

Companion Products

| NEB # | PRODUCT | VOLUME |
|--------|---------------------------------|----------|
| T2010S | Monarch® Total RNA Miniprep Kit | 50 preps |

NEBNext ARTIC Human Primers

| PRIMER MIX | GENE | POSITION | PRIMERS |
|--|-------|-----------------|--|
| NEBNext ARTIC Human Control Primer Pairs 1 | EDF1 | 113 bp – 501 bp | GGCCAAATCCAAGCAGGCTA GTGTTTCATTTTCGCCCTAGGC |
| NEBNext ARTIC Human Control Primer Pairs 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 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

Checklist

1. cDNA Synthesis

- ☐ 1.1. Setup cDNA synthesis reactions, mix, and spin down:
 - ☐ 8 µl RNA Sample (OR Nuclease-free Water for no template control)
 - ☐ 2 µl LunaScript RT SuperMix
- ☐ 1.2. Thermal cycle (Heated lid to 105°C; 2 min 25°C, 20 min 55°C, 1 min 95°C, hold at 4°C)

2. Targeted cDNA Amplification

If using the Human control primers (NEBNext ARTIC Human Control Primer Pairs 1 and 2) skip to Step 2.6.

- ☐ 2.1.A.Setup, mix, and spin down targeted cDNA amplification reactions, Pool Set A:
 - ☐ 4.5 µl cDNA sample
 - ☐ 6.25 µl Q5 Hot Start High-Fidelity Master Mix
 - ☐ 1.75 µl ARTIC SARS-CoV-2 Primer Mix 1
- ☐ 2.1.B.Setup, mix, and spin down targeted cDNA amplification reactions, Pool Set B:
 - ☐ 4.5 µl cDNA sample
 - ☐ 6.25 µl Q5 Hot Start High-Fidelity Master Mix
 - ☐ 1.75 µl ARTIC SARS-CoV-2 Primer Mix 2
- ☐ 2.2. Thermal cycle (Heated lid 105°C; 98°C 30 sec, 35 cycles of 95°C for 15 sec and 63°C for 5 min, 65°C for 5 min, hold at 4°C).
- ☐ 2.3. Combine the Pool A and Pool B PCR reactions for each sample.
- ☐ 2.5. Skip to Step 3, cleanup of cDNA amplicons.
- ☐ 2.6. If using a 24 reaction kit
 - ☐ 42 µl ARTIC SARS-CoV-2 Primer Mix 1
 - ☐ 0.7 µl NEBNext ARTIC Human Control Primer Pairs 1

If using a 96 reaction kit

 - ☐ 168 µl ARTIC SARS-CoV-2 Primer Mix 1
 - ☐ 2.8 µl NEBNext ARTIC Human Control Primer Pairs 1
- ☐ 2.7. If using the optional Human Primer Mix 2, combine, mix and spin down ARTIC SARS-CoV-2 Primer Mix 2 and NEBNext ARTIC Human Control Primer Pairs 2
 - If using a 24 reaction kit

 - ☐ 42 µl ARTIC SARS-CoV-2 Primer Mix 2
 - ☐ 0.7 µl NEBNext ARTIC Human Control Primer Pairs 2
 - If using a 96 reaction kit

 - ☐ 168 µl ARTIC SARS-CoV-2 Primer Mix 2
 - ☐ 2.8 µl NEBNext ARTIC Human Control Primer Pairs 2
- ☐ 2.8. Setup, mix, and spin down targeted cDNA amplification reactions, Pool Set A:
 - ☐ 4.5 µl cDNA sample
 - ☐ 6.25 µl Q5 Hot Start High-Fidelity Master Mix
 - ☐ 1.75 µl of the combined ARTIC SARS-CoV-2 Primer Mix 1 and NEBNext ARTIC Human Control Primer Pairs 1

- [_] 2.9. Setup, mix, and spin down targeted cDNA amplification reactions, Pool Set B:
- [_] 4.5 µl cDNA sample
 - [_] 6.25 µl Q5 Hot Start High-Fidelity Master Mix
 - [_] 1.75 µl of the combined ARTIC SARS-CoV-2 Primer Mix 2 and NEBNext ARTIC Human Control Primer Pairs 2
- [_] 2.10. Thermal cycle (Heated lid 105°C; 98°C 30 sec, 35 cycles of 95°C for 15 sec and 63°C for 5 min, 65°C for 5 min, hold at 4°C)
- [_] 2.11. Combine the Pool A and Pool B PCR reactions for each sample

3. Cleanup of cDNA Amplicons

- [_] 3.1. Vortex beads
- [_] 3.2. Add 20 µl of beads to sample and mix by pipetting 10 times
- [_] 3.3. Incubate for 5 min
- [_] 3.4. Place tubes on magnet
- [_] 3.5. Wait 5 min and remove supernatant (keep the beads)
- [_] 3.6. On magnet add 200 µl 80% ethanol, wait 30 seconds and remove
- [_] 3.7. Repeat Step 3.6 once
- [_] 3.8. Air dry beads, do not overdry
- [_] 3.9. Off magnet add 16 µl 10 mM Tris-HCl or 0.1X TE, OR 15 µl if not assessing amplicons
- [_] 3.10. Mix by pipetting 10 times and incubate 2 min
- [_] 3.11. Place tubes on magnet, wait 5 min, and transfer 14 µl to a new tube, OR 13 µl if not assessing amplicons

4. Fragmentation/End Prep

- [_] 4.1. Thaw, vortex, and quick spin FS Reaction Buffer
- [_] 4.2. Vortex FS Enzyme Mix for 5-8 sec, quick spin
- [_] 4.3. Add FS Reagents to sample:
- [_] 3.5 µl FS Reaction Buffer
 - [_] 1 µl FS Enzyme Mix
- [_] 4.4. Vortex mx for 5 sec, quick spin
- [_] 4.5. Thermal cycle (Heated lid \geq 75°C; 30 min 37°C, 30 min 65°C, hold at 4°C)

5. Adaptor Ligation

- [_] 5.1. Add Ligation reagents to sample:
- [_] 1.25 µl diluted adaptor
 - [_] 15 µl Ligation Master Mix
- [_] 5.2. Pipette mix 10 times with pipette set to 40 µl, quick spin
- [_] 5.3. Incubate 15 min at 20°C (heated lid off)
- [_] 5.4. Add 1.5 µl USER
- [_] 5.5. Pipette mix 10 times with pipette set to 40 µl, quick spin; incubate 15 min 37°C (heated lid \geq 47°C)

6. Cleanup of Adaptor-ligated DNA

- ☐ 6.1. Vortex beads
- ☐ 6.2. Add 28 µl of beads to sample and mix by pipetting 10 times
- ☐ 6.3. Incubate for 5 min
- ☐ 6.4. Place tubes on magnet
- ☐ 6.5. Wait 5 min and remove supernatant (keep the beads)
- ☐ 6.6. On magnet add 200 µl 80% ethanol, wait 30 seconds and remove
- ☐ 6.7. Repeat Step 6.6 once
- ☐ 6.8. Air dry beads, do not overdry
- ☐ 6.9. Off magnet add 10 µl 0.1X TE
- ☐ 6.10. Mix by pipetting 10 times and incubate 2 min
- ☐ 6.11. Place tubes on magnet, wait 5 min, and transfer 7.5 µl to a new tube

7. PCR Enrichment of Adaptor-ligated DNA

7.1. PCR Amplification

- 7.1.1. Add PCR Reagents to sample
 - 7.1.1A. ☐ A (12.5 µl Library PCR Master Mix, 2.5 µl index primer/ i7 primer; 2.5 µl universal primer/ i5 primer) **OR**
 - 7.1.1B. ☐ B (12.5 µl Library PCR Master Mix, 5 µl index and universal primer)
- ☐ 7.1.2. Pipette mix 10 times with pipette set to 20 µl, quick spin
- ☐ 7.1.3. Thermal cycle (Heated lid to 105°C; 98°C 30 sec, 5 cycles of 98°C for 10 sec and 65°C for 75 sec, 65°C for 5 min, hold at 4°C)

8. Cleanup of PCR Reaction

- ☐ 8.1. Vortex beads
- ☐ 8.2. Add 22.5 µl of beads to sample and mix by pipetting 10 times
- ☐ 8.3. Incubate for 5 min
- ☐ 8.4. Place tubes on magnet
- ☐ 8.5. Wait 5 min and remove supernatant (keep the beads)
- ☐ 8.6. On magnet add 200 µl 80% ethanol, wait 30 seconds and remove
- ☐ 8.7. Repeat Step 8.6 once
- ☐ 8.8. Air dry beads, do not overdry
- ☐ 8.9. Off magnet add 17 µl 10 mM Tris-HCl or 0.1X TE
- ☐ 8.10. Mix by pipetting 10 times. Incubate 2 min.
- ☐ 8.11. Place tubes on magnet. Wait 5 min. and transfer 15 µl to a new tube
- ☐ 8.12. Check size distribution on Bioanalyzer or TapeStation

Revision History

| REVISION # | DESCRIPTION | DATE |
|------------|-------------|------|
| 1.0 | N/A | 2/21 |

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New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054 Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com