

# NEBNext<sup>®</sup> ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies<sup>®</sup>)

NEB #E7660S/L

24/96 reactions

Version 1.0\_2/21

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## The Kit Includes

*The volumes provided are sufficient for preparation of up to 24 barcoding reactions (NEB #E7660S, minimum 6 barcoding samples per run for total 4 runs) and 96 barcoding reactions (NEB #E7660L, minimum 24 barcoding samples per run for total 4 runs). Colored bullets represent the color of the cap of the tube containing the reagent. If one plans to follow a different protocol, additional reagents can be purchased separately)*

### Package 1: Store at -20°C.

- (lilac) LunaScript<sup>®</sup> RT SuperMix (5X)
- (lilac) Q5<sup>®</sup> Hot Start High-Fidelity 2X Master Mix
- (green) NEBNext Ultra II End Prep Enzyme Mix
- (green) NEBNext Ultra II End Prep Reaction Buffer
- (red) Blunt/TA Ligase Master Mix
- (red) NEBNext Quick T4 DNA Ligase
- (red) NEBNext Quick Ligation Reaction Buffer (5X)
- (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
- (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), 13-24 (EXP-NBD114) or 1-96 (EXP-NBD196)
- 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 #S1515, 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)

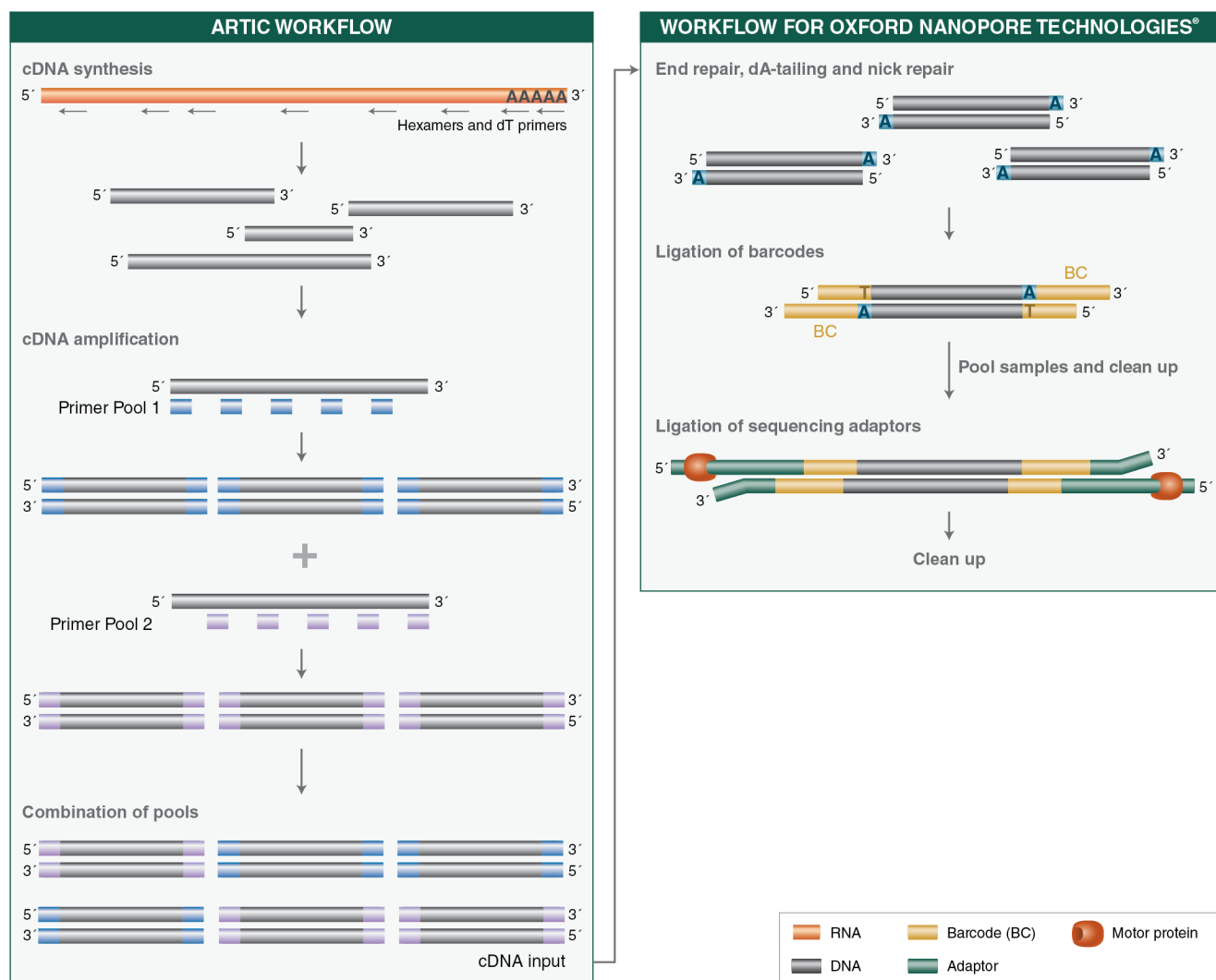
## Overview

The NEBNext ARTIC SARS-CoV-2 Companion Library Prep Kit (Oxford Nanopore Technologies) 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 Oxford Nanopore platform. Primers targeting the human EDF1 and NEDD8 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 Oxford Nanopore 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 Companion Library Prep Kit for Oxford Nanopore.**



## 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  $\geq 10$  copies of the (SARS-CoV-2) viral genome as input. In addition, we recommend setting up a no template control reaction and **all reactions are set-up in a hood.**

### 1. cDNA Synthesis

The presence of genomic DNA or 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 10 times by pipetting and spin down the LunaScript RT SuperMix reagents (contains primers). Prepare the cDNA synthesis reaction as described below:

COMPONENT	VOLUME
RNA Sample*	8 $\mu$ l
● (lilac) LunaScript RT SuperMix	2 $\mu$ l
Total Volume	10 $\mu$ l

\*Up to 0.5  $\mu$ g total RNA can be used in a 10  $\mu$ l reaction.

- 1.2. Flick the tube or pipet up and down to mix followed by a quick spin.  
1.3. For no template controls, mix the following components

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

- 1.4. Flick the tube or pipet up and down to mix followed by a quick spin  
1.5. Incubate reactions in a thermocycler with lid temperature at 105°C 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	

\*Set heated lid to 105°C



**Samples can be stored at –20°C if they are not used immediately.**

## 2. Targeted cDNA Amplification

**Note:** cDNA from 2 µl to 4.5 µl can be used as input. When using less than 4.5 µl of cDNA, add nuclease-free water to a final cDNA 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 ARTIC Primer Pool should be combined prior to use. More specifically, NEBNext ARTIC Human Control Primer Pairs 1 should be combined with ARTIC Primer Pool 1 and NEBNext ARTIC Human Control Primer Pairs 2 with ARTIC Primer Pool 2. Mixing directions are listed below.

- 2.1. Gently mix Q5 Hot Start High Fidelity 2X MM 10 times by pipetting and spin down reagents. Prepare the split pool 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.

- 2.2. Flicking the tube or pipetting up and down to mix followed by a quick spin.

### 2.3. 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 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.

- 2.4. Flicking the tube or pipetting up and down to mix followed by a quick spin.

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

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

\* Q5 Set heated lid to 105°C.



Samples can be stored at –20°C if they are not used immediately.

### 3. Cleanup of cDNA Amplicons

If prefer to omit the cleanup step, please follow the newer version of nCoV-2019 sequencing protocol v3 (LoCost)

<https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye/abstract>



**Note:** SPRIselect or AMPure® XP Beads can be used as well. If using AMPure XP Beads, allow the beads to warm to room temperature for at least 30 minutes before use. These bead 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.

- 3.1. Combine pool A and pool B PCR Reactions.
- 3.2. Vortex SPRIselect or NEBNext Sample Purification Beads to resuspend.
- 3.3. Add 20 µl (0.8X) resuspended beads to the combined PCR reaction. Mix well by flicking the tube or pipetting up and down to mix and a very short 2-3 seconds quick centrifugation. Be sure to stop the centrifugation before the beads start to settle out.
- 3.4. Incubate samples at room temperature for 10 minutes.
- 3.5. Place the tubes on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample 3 seconds to collect the liquid from the sides of the tube before placing on the magnetic stand.
- 3.6. After 2 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**).
- 3.7. Add 200 µl of 80% freshly prepared ethanol to the tube 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.
- 3.8. Repeat Step 3.7. once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube for 3 seconds, place back on the magnetic stand and remove traces of ethanol with a p10 pipette tip.
- 3.9. Air dry the beads for 30 seconds while the tube 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. When the beads turn lighter brown and start to crack, they are too dry.**

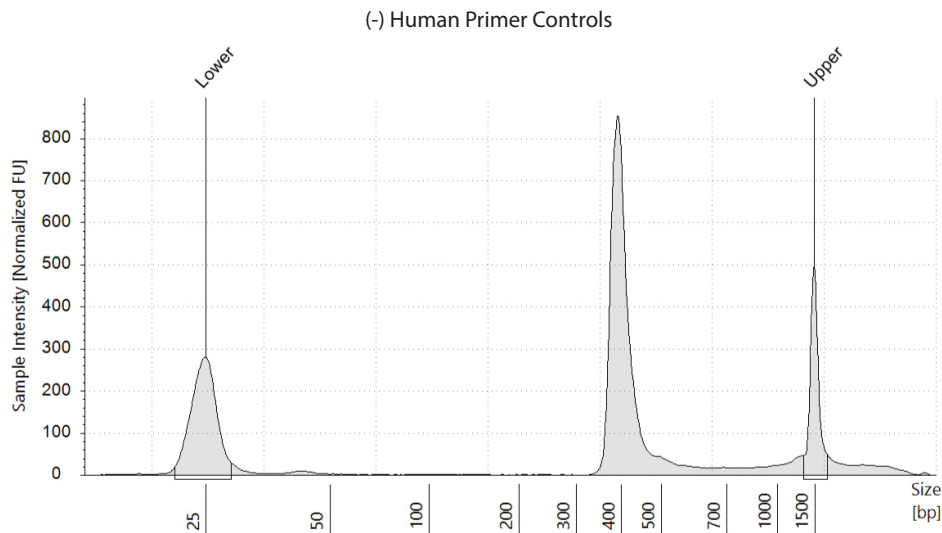
- 3.10. Remove the tube from the magnetic stand. Elute the DNA target from the beads by adding 21 µl Nuclease-free water.
- 3.11. Mix well by flicking the tube or pipetting up and down to mix and followed by a very short centrifugation. Incubate for 10 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.
- 3.12. Place the tube on the magnetic stand. After 2 minutes (or when the solution is clear), transfer 20 µl to PCR tubes.
- 3.13. We recommend assessing cDNA concentrations with a Qubit fluorometer. Amplicons may also be run on a Bioanalyzer® or a TapeStation to confirm 400 bp size of amplicons.



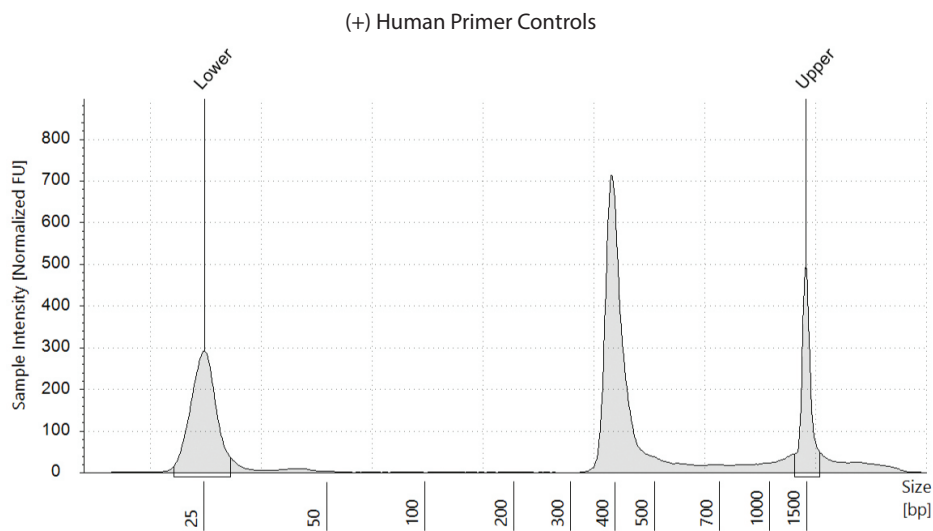
**Samples can be stored at –20°C if they are not used immediately.**

**Figure 3.13: Example of cDNA amplicons generated from 1000 genome copies of SARS CoV-2 in the absence (A) and presence (B) of human primer controls.**

**A**



**B**



#### 4. NEBNext End Prep

- 4.1. Dilute each amplicon sample (3.12.) into 50 ng/12.5  $\mu$ l concentration using Nuclease-free water. Add the following components to a PCR tube (End Prep Reaction and Buffer can be pre-mixed and stable on ice for 4 hours):

COMPONENT	VOLUME
Targeted cDNA Amplicons (3.12)	12.5 $\mu$ l
● (green) NEBNext Ultra II End Prep Reaction Buffer	1.75 $\mu$ l
● (green) NEBNext Ultra II End Prep Enzyme Mix	0.75 $\mu$ l
Total Volume	15 $\mu$ l

- 4.2 Flick the tube or pipet up and down to mix the solution. Perform a quick spin to collect all liquid from the sides of the tube.

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

- 4.3 Place in a thermocycler, with the heated lid set to  $\geq 75^{\circ}\text{C}$ , and run the following program:
- 10 minutes @  $20^{\circ}\text{C}$
- 10 minutes @  $65^{\circ}\text{C}$
- Hold at  $4^{\circ}\text{C}$



**If necessary, samples can be stored at  $-20^{\circ}\text{C}$  for a few days; however, a slight loss in yield ( $\sim 20\%$ ) may be observed. We recommend continuing with barcode ligation before stopping.**

## 5. Barcode Ligation

- 5.1. Add the following components directly to a sterile nuclease-free PCR tube:

COMPONENT	VOLUME
○ (white) Nuclease-free water	6 $\mu\text{l}$
End-prepped DNA (4.3.)	1.5 $\mu\text{l}$
Native Barcode*	2.5 $\mu\text{l}$
● (red) Blunt/TA Ligase Master Mix**	10 $\mu\text{l}$
Total Volume	20 $\mu\text{l}$

\* Native Barcodes are provided in Oxford Nanopore Technologies Native Barcoding Expansion 1-12 (EXP-NBD104) and 13-24 (EXP-NBD114) or 1-96 (EXP-NBD196)

\*\* Mix the Blunt/TA Ligase Master Mix by pipetting up and down several times prior to adding to the reaction

- 5.2. Flick the tube or pipet up and down to mix solution. Perform a quick spin to collect all liquid from the sides of the tube. **(Caution: The Blunt/TA Ligase 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 room temperature for 20 minutes followed by  $65^{\circ}\text{C}$  for 10 minutes. On ice for 1 min.
- 5.4. Pool all barcoded samples into a 1.5 ml DNA LoBind Tube.

## 6. Cleanup of Barcoded cDNA

The following section is for cleanup of the ligation reaction.

**Note: The volumes of SPRIselect or NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step. AMPure XP Beads can be used as well. If using AMPure XP Beads, allow the beads to warm to room temperature for at least 30 minutes before use.**

- 6.1. Vortex NEBNext Sample Purification Beads to resuspend.
- 6.2. Add 0.4X resuspended beads to pooled barcode samples (5.4.) (for example, 192  $\mu\text{l}$  of resuspended beads should be added to a pool of 24 barcoded samples where the sample volume is 480  $\mu\text{l}$ ). Mix well by flicking the tube. Perform a quick spin for 3 seconds to collect all liquid from the sides of the tube.
- 6.3. Incubate samples on bench top for 10 minutes at room temperature.
- 6.4. Place the tube on a 1.5 ml magnetic stand (such as NEB S1506) 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 2 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. Wash the beads by adding 250  $\mu\text{l}$  of Short Fragment buffer (SFB). Flick the tube or pipet up and down to mix to resuspend pellet. If necessary, quickly spin the sample for 3 seconds to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.
- 6.7. Place the tube on an appropriate magnetic stand for 2 minutes (or when the solution is clear) to separate the beads from the supernatant. Remove the supernatant.
- 6.8. Repeat Step 6.6. and 6.7. once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube, place back on the magnetic stand and remove traces of SFB with a p10 pipette tip



- 6.9. Add 200 µl of 80% freshly prepared ethanol to the tube while on 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.
- 6.10. A quick spin and place the sample tube on the magnetic stand, remove any residual ethanol.
- 6.11. Air dry the beads for 30 seconds while the tube 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.**
- 6.12. Remove the tube from the magnetic stand. Elute the DNA target from the beads by adding 33 µl of Nuclease-free water.
- 6.13. Resuspend the pellet by flicking the tube or pipetting up and down to mix. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample for 3 seconds to collect the liquid from the sides of the tube before placing back on the magnetic stand.
- 6.14. Place the tube on the magnetic stand. After 2 minutes (or when the solution is clear), transfer 32 µl to a new 1.5 ml Eppendorf DNA LoBind Tube.
- 6.15. We recommend assessing cDNA concentrations with a Qubit fluorometer



Samples can be stored at –20°C if they are not used immediately.

## 7. Adapter Ligation

- 7.1. Add the following components into a 1.5 ml Eppendorf DNA LoBind Tube

COMPONENT	VOLUME
Native barcoded and purified DNA (6.14.)	30 µl
Adapter Mix II (AMII)**	5 µl
● (red) NEBNext Quick Ligation Reaction Buffer (5X)	10 µl
● (red) NEBNext Quick T4 Ligase	5 µl
Total Volume	50 µl

\* Mix the NEBNext Quick Ligation Reaction Buffer by pipetting up and down several times prior to adding to the reaction.

\*\* Adapter Mix II is provided by Oxford Nanopore Technologies Native Barcoding Expansion 1-12 (EXP-NBD104), 13-24 (EXP-NBD114) and 1-96 (EXP-NBD-196) kits.

- 7.2. Flick the tube or pipet up and down to mix solution. Perform a quick spin for 3 seconds to collect all liquid from the sides of the tube. **(Caution: The NEBNext Quick Ligation Buffer is 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).**
- 7.3. Incubate at room temperature for 20 minutes.
- 7.4. Proceed to Cleanup of Adapter-ligated cDNA in Section 8.

## 8. Cleanup of Adapter Ligated DNA

**Note: The volumes of SPRIselect or NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step. AMPure XP beads can be used as well. If using AMPure XP beads, allow the beads to warm to room temperature for at least 30 minutes before use. These volumes may not work properly for a cleanup at a different step in the workflow.**

- 8.1. Vortex NEBNext Sample Purification Beads to resuspend.
- 8.2. Add 50 µl (1X) resuspended beads to the ligation mix. Mix well by flicking the tube or pipetting up and down to mix followed by a quick spin for 3 seconds.
- 8.3. Incubate samples for 10 minutes at room temperature.
- 8.4. Place the tube 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 2 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. Wash the beads by adding 250 µl of Short Fragment Buffer (SFB). Flick the tube or pipet up and down to mix to resuspend pellet. 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. Place the tube on an appropriate magnetic stand.
- 8.7. Wait for 2 minutes (or when the solution is clear) to separate the beads from the supernatant. Remove the supernatant.
- 8.8. Repeat Step 8.6. and 8.7. 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 SFB with a p10 pipette tip.
- 8.9. Remove the tube from the magnetic stand. Elute the DNA target from the beads by adding 15 µl of Elution Buffer (EB) provided in SQK-LSK109 kit from Oxford Nanopore.
- 8.10. Resuspend the pellet well in EB buffer by flicking the tube or pipetting up and down to mix. Incubate for 10 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 2 minutes (or when the solution is clear), transfer 15 µl to a new DNA LoBind tube.
- 8.12. Use Qubit to quantify 1 µl DNA sample. Follow Oxford Nanopore Protocol SQK-LSK109 to prepare MinION® flow cell and sequencing mix using up to 15 ng adapter-ligated cDNA sample (8.11).

## Kit Components

### NEB #E7660S Table of Components

NEB #	PRODUCT	VOLUME
E7651A	LunaScript RT SuperMix (5X)	0.048 ml
E7652A	Q5 Hot Start High-Fidelity 2X Master Mix	0.30 ml
E7661A	NEBNext Ultra II End Prep Enzyme Mix	0.018 ml
E7662A	NEBNext Ultra II End Prep Reaction Buffer	0.042 ml
E7663A	Blunt/TA Ligase Master Mix	0.24 ml
E7664A	NEBNext Quick T4 DNA Ligase	0.020 ml
E7665A	NEBNext Quick Ligation Reaction Buffer	0.040 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	0.007 ml
E7728A	NEBNext ARTIC Human Control Primer Pairs 2	0.007 ml
E7667A	Nuclease free-Water	1.50 ml
E7666S	NEBNext Sample Purification Beads	0.872 ml

### NEB #E7660L Table of Components

NEB #	PRODUCT	VOLUME
E7651AA	LunaScript RT SuperMix (5X)	0.192 ml
E7652AA	Q5 Hot Start High-Fidelity 2X Master Mix	1.2 ml
E7661AA	NEBNext Ultra II End Prep Enzyme Mix	0.072 ml
E7662AA	NEBNext Ultra II End Prep Reaction Buffer	0.168 ml
E7663AA	Blunt/TA Ligase Master Mix	0.96 ml
E7664A	NEBNext Quick T4 DNA Ligase	0.020 ml
E7665A	NEBNext Quick Ligation Reaction Buffer	0.040 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	0.007 ml
E7728A	NEBNext ARTIC Human Control Primer Pairs 2	0.007 ml
E7667AA	Nuclease free-Water	4.7 ml
E7666L	NEBNext Sample Purification Beads	2.90 ml

## NEBNext ARTIC Human Primers

PRIMER MIX	GENE	POSITION	PRIMERS
NEBNext ARTIC Human Control Primer Pairs 1	EDF1	113 bp – 501 bp	GGCCAAATCCAAGCAGGCTA GTGTTTCATTTCGCCCTAGGC
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 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](https://github.com/joshquick/artic-ncov2019/blob/master/primer_schemes/nCoV-2019/V3/nCoV-2019.tsv)

## Checklist

### 1. cDNA Synthesis

- ☐ 1.1. Add cDNA Synthesis Reagent to 8 µl RNA sample:
  - ☐ 2 µl (lilac) LunaScript RT SuperMix
  - ☐ 8 µl RNA sample
- ☐ 1.2. Flick the tube or pipet up and down to mix, quick spin
- ☐ 1.3. Set up No Template Control:
  - ☐ 2 µl (lilac) LunaScript RT SuperMix
  - ☐ 8 µl Nuclease free-Water
- ☐ 1.4. Flick the tube or pipet up and down to mix, quick spin
- ☐ 1.5. Thermal cycle (Heated lid  $\geq 105^{\circ}\text{C}$ ;  $25^{\circ}\text{C}$  for 2 mins,  $55^{\circ}\text{C}$  for 20 mins,  $95^{\circ}\text{C}$  for 1 min)

### 2. Targeted cDNA Amplification

- ☐ 2.1. Add Targeted cDNA Amplification Reagents to 4.5 µl sample (1.5.), Pool Set A:
  - ☐ 4.5 µl cDNA sample (1.5.)
  - ☐ 6.25 µl (lilac) Q5 Hot Start High-Fidelity 2X MM
  - ☐ 1.75 µl (lilac) ARTIC SARS-CoV2 Primer Mix 1 (11 µM)
- ☐ 2.2. Flick the tube or pipet up and down to mix, quick spin
- ☐ 2.3. Add Targeted cDNA Amplification Reagents to 4.5 µl sample (1.5.), Pool Set B:
  - ☐ 4.5 µl cDNA sample (1.5.)
  - ☐ 6.25 µl (lilac) Q5 Hot Start High-Fidelity 2X MM
  - ☐ 1.75 µl (lilac) ARTIC SARS-CoV2 Primer Mix 2 (11 µM)
- ☐ 2.4. Flick the tube or pipet up and down to mix, quick spin
- ☐ 2.5. Thermal cycle (Heated lid  $\geq 105^{\circ}\text{C}$ ;  $98^{\circ}\text{C}$  for 30 sec, 35 cycles of  $95^{\circ}\text{C}$  for 15 sec and  $63^{\circ}\text{C}$  for 5 mins) Hold at  $4^{\circ}\text{C}$ )

### 3. Cleanup of cDNA Amplicons

- ☐ 3.1. Combine Pool Set A and Pool Set B PCR reactions, one sample per tube.
- ☐ 3.2. Vortex NEBNext Sample Purification Beads to resuspend.
- ☐ 3.3. Add 20 µl of resuspended beads to sample and mix by flicking the tube or pipetting up and down to mix followed by a quick spin.
- ☐ 3.4. Incubate for 10 mins at room temperature
- ☐ 3.5. Place tubes on the magnetic stand
- ☐ 3.6. Wait 2 min and remove supernatant (keep the beads)
- ☐ 3.7. On magnetic stand add 200 µl 80% ethanol to the tube, wait 30 seconds and remove supernatant
- ☐ 3.8. Repeat Step 3.7. once. If necessary, briefly spin the tube, place back on the magnetic stand and remove traces of ethanol
- ☐ 3.9. Air dry beads for 30 seconds with lid open, do not over-dry
- ☐ 3.10. Off magnet add 21 µl Nuclease-free water to the tube
- ☐ 3.11. Flicking the tube or pipetting up and down to mix to resuspend the beads pellet followed by a quick spin. Incubate at room temperature for 2 mins
- ☐ 3.12. Place tubes on the magnetic stand. Wait 2 mins or when the solution is clear and transfer 20 µl to PCR tubes
- ☐ 3.13. Use Qubit fluorometer to quantitate the sample concentration

#### 4. NEBNext End Prep

- ☐ 4.1. Dilute each amplicon sample (3.12.) into 50 ng/12.5 µl concentration using Nuclease-free water. Add End Prep Reagents to sterile nuclease-free PCR tube:
  - ☐ 12.5 µl of Targeted cDNA Amplicons (4.1.)
  - ☐ 1.75 µl ● (green) NEBNext Ultra II End Prep Reaction Buffer
  - ☐ 0.75 µl ● (green) NEBNext Ultra II End Prep Enzyme Mix
- ☐ 4.2. Flick the tube or pipet up and down to mix, quick spin
- ☐ 4.3. Thermal cycle (Heated lid = 75°C; 20°C for 10 mins, 65°C for 10 mins, hold at 4°C)

#### 5. Barcode Ligation

- ☐ 5.1. Add following reagents to a sterile nuclease-free PCR tube:
  - ☐ 6 µl Nuclease-free water
  - ☐ 1.5 µl End Prepped cDNA (4.3)
  - ☐ 2.5 µl Native Barcode
  - ☐ 10 µl ● (red) Blunt/TA Ligase Master Mix
- ☐ 5.2. Flick the tube or pipet up and down to mix, quick spin
- ☐ 5.3. Incubate the reaction mix at room temperature for 20 mins followed by 65°C for 10 mins, on ice 1 min
- ☐ 5.4. Pool all barcoded samples into a 1.5 ml DNA LoBind tube

#### 6. Cleanup of Barcoded cDNA

- ☐ 6.1. Vortex NEBNext Sample Purification Beads to resuspend
- ☐ 6.2. Add 0.4X resuspended beads to barcoded sample pool (5.4.). Flick tube to mix, a quick spin
- ☐ 6.3. Incubate for 10 mins at room temperature
- ☐ 6.4. Place tubes on the magnetic stand
- ☐ 6.5. Wait 2 min and remove supernatant (keep the beads)
- ☐ 6.6. Wash the beads with 250 µl of Short Fragment Buffer solution (SFB, Oxford Nanopore. SQK-LSK109). Flick the tube or pipet up and down to mix to resuspend pellet, quick spin
- ☐ 6.7. Placing tube on the magnetic stand. Wait 2 mins or when the solution is clear, remove the supernatant.
- ☐ 6.8. Repeat Step 6.6. and 6.7. once. A quick spin, back on the magnetic stand and remove any residual SFB.
- ☐ 6.9. On magnetic stand add 200 µl 80% ethanol to the tube and remove ethanol without disturbing the pellet
- ☐ 6.10. A quick spin and place tube on the magnetic stand. Remove any residual ethanol
- ☐ 6.11. Air dry beads for 30 seconds with lid open
- ☐ 6.12. Off magnet add 33 µl Nuclease-free water
- ☐ 6.13. Flick the tube or pipet up and down to mix, a quick spin and incubate sample tube at room temperature for 2 mins
- ☐ 6.14. Place tube on the magnetic stand. Wait 2 mins or when solution is clear and transfer 32 µl to a 1.5 ml DNA LoBind tube
- ☐ 6.15. Use Qubit fluorometer to quantitate the sample concentration

#### 7. Adapter Ligation

- ☐ 7.1. Add following reagents to a LoBind DNA tube:
  - ☐ 30 µl barcoded and cleaned cDNA (6.14).
  - ☐ 5 µl Adapter Mix II (AMII).
  - ☐ 10 µl ● (red) NEBNext Quick Ligation Reaction Buffer.
  - ☐ 5 µl ● (red) NEBNext Quick T4 Ligase.
- ☐ 7.2. Flick the tube or pipet up and down to mix and a quick spin.

[ \_ ] 7.3 Incubate the reaction mix at room temperature for 20 mins.

[ \_ ] 7.2. Proceed to Cleanup of adapter -ligated cDNA in Section 8.

## 8. Cleanup of Adapter Ligated cDNA

[ \_ ] 8.1. Vortex NEBNext Sample Purification Beads.

[ \_ ] 8.2. Add 50 µl (1X) of beads to sample. Flick to mix and a quick spin.

[ \_ ] 8.3. Incubate for 10 mins at room temperature.

[ \_ ] 8.4. A quick spin and place sample tube on the magnetic stand.

[ \_ ] 8.5. Wait 2 mins and remove supernatant (keep the beads).

[ \_ ] 8.6. On magnet add 250 µl Short Fragment Buffer. Flicking the tube or pipetting up and down to mix to resuspend the pellet followed by a quick spin.  
Place tube back to the magnetic stand.

[ \_ ] 8.7. Wait 2 mins and remove supernatant (keep the beads).

[ \_ ] 8.8. Repeat Step 8.6.–8.7. once. A quick spin and place tube on the magnetic stand. Remove any residual SFB buffer.

[ \_ ] 8.9. Off magnet add 15 µl Elution Buffer (EB, Oxford Nanopore. SQK-LSK109).

[ \_ ] 8.10. Flick the tube or pipet up and down to mix to resuspend the beads pellet and incubate for 10 mins at room temperature.

[ \_ ] 8.11. Place tubes on the magnetic stand. Wait 2 min and transfer 15 µl supernatant to a new DNA LoBind tube.

[ \_ ] 8.12. Use Qubit fluorometer to quantitate the sample concentration. Follow Oxford Nanopore protocol SQK-LSK109 (or SQK-LSK111) to prepare MinION flow cell and loading library mix containing up to 15 ng adapter-ligated cDNA sample.

## Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	2/21

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