Version 4

May 25, 2021

NEBNext® ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies®) E7660 V.4

New England Biolabs[1]

[1]New England Biolabs

dx.doi.org/10.17504/protocols.io.budxns7n

New England Biolabs (NEB) Coronavirus Method Development Community

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ABSTRACT

This protocol details methods for the NEBNext® ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies®), NEB #E7660S/L 24/96 reactions.

Standard Protocol with PCR Bead Cleanup: This protocol includes a cleanup and normalization step for each sample after cDNA synthesis. Performing the cleanup and normalization step creates library pools where the reads for each library are more evenly distributed. These pools will likely achieve sufficient and equal coverage in less run time, but they take more hands-on time.

There is also the **Express Protocol without PCR Bead Cleanup** (This protocol does not include a cleanup and normalization step for each sample after cDNA synthesis. Performing the cleanup and normalization step creates library pools where the reads for each library are more evenly distributed. Skipping these steps reduces hands on time, but may require a longer sequencing run to obtain sufficient coverage for each sample.
Please look for "E7660" on https://www.protocols.io/

DOI

dx.doi.org/10.17504/protocols.io.budxns7n

EXTERNAL LINK

https://www.neb.com/-/media/nebus/files/manuals/manuale7660.pdf?rev=48c42313dcb64b0dbb16c4bfd1563a27

PROTOCOL CITATION

New England Biolabs 2021. NEBNext® ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies®) E7660. **protocols.io**
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GUIDELINES

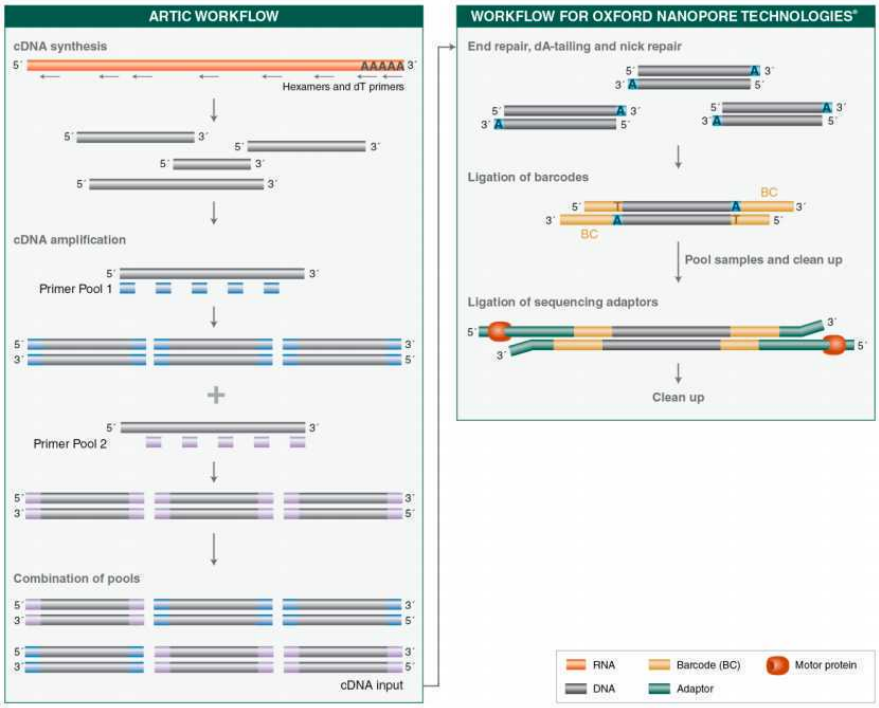
Overview

The NEBNext ARTIC SARS-CoV-2 Companion Library Prep Kit (Oxford Nanopore Technologies) contains the enzymes, buffers, beads 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.



MATERIALS TEXT

The Library 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). If one plans to follow a different protocol, additional reagents can be purchased separately).

Package 1: Store at –20°C.

(lilac) LunaScript® RT SuperMix
(lilac) Q5® 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 Ligase
(red) NEBNext Quick Ligation Reaction Buffer

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(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) and 13-24 (EXP-NBD114)
- 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)

Kit Components
NEB #E7660S Table of Components

A	B	C
NEB #	PRODUCT	VOLUME
E7651A	LunaScript RT SuperMix (5X)	0.048 ml
E7652A	Q5 Hot Start High-Fidelity 2X Master Mix	0.30 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

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A	B	C
NEB #	PRODUCT	VOLUME
E7651AA	LunaScript RT SuperMix (5X)	0.192 ml
E7652AA	Q5 Hot Start High-Fidelity 2X Master Mix	1.2 ml
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

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

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

NEBNext ARTIC SARS-CoV-2 Primers

NEBNext ARTIC SARS-CoV-2 Primers for SARS-CoV-2 genome amplification are based on hCoV-2019/nCoV-2019 Version 3 (v3) sequences with balanced primer concentrations. Sequence information can be found at: https://github.com/joshquick/artic-ncov2019/blob/master/primer_schemes/nCoV-2019/V3/nCoV-2019.tsv

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

BEFORE STARTING

Note: The amount of RNA required for detection depends on the abundance of the RNA of interest. In general, we recommend, using > 10 copies of the (SARS-CoV-2) viral genome as input. In addition, we recommend setting up a no template control reaction and **all reactions are set-up in a hood**.

Also note: If sample Ct is between 12-15, then it is recommended per the ARTIC network nCoV 2019 sequencing protocol v3 LoCost to dilute the sample 100-fold in water, if between 15-18 then dilute 10-fold in water. This will reduce the likelihood of PCR-inhibition.

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

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Gently mix 10 times by pipetting and spin down the LunaScript RT SuperMix reagents (contains primers). 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

*Up to 0.5 µg total RNA can be used in a 10 µl reaction.

2 

Flick the tube or pipet up and down 10 times to mix followed by a quick spin.

3 

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

4 

Flick the tube or pipet up and down 10 times to mix followed by a quick spin.

5 

Incubate reactions in a thermocycler with lid temperature at 105°C with the following steps:

A	B	C	D
CYCLE STEP	TEMP	TIME	CYCLE
Primer Annealing	25°C	2 minutes	1
cDNA Synthesis	55°C	20 minutes	
Heat Inactivation	95°C	1 minute	
Hold	4°C	∞	

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

Targeted cDNA Amplification

6

4.5 µl cDNA input is recommended. If using less than 4.5 µl of cDNA, add nuclease-free water to

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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.

7

Gently mix Q5 Hot Start High Fidelity 2X master mix 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 Primer Pairs and a *24 reaction kit*, combine

0.7 µl NEBNext ARTIC Human Control Primer Pairs 1 with

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

168 µl NEBNext ARTIC SARS-CoV-2 Primer Mix 1 , vortex and spin down reagents. Use

1.75 µl combined mix for each Pool Set A reaction.

A	B
COMPONENT	VOLUME
cDNA (Step 5)	4.5 µl
(lilac) Q5 Hot Start High-Fidelity 2X MM	6.25 µl
NEBNext 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 NEBNext 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 NEBNext ARTIC Human Control Primer Pairs 2 with

42 µl NEBNext ARTIC SARS-CoV-2 Primer Mix 2 , vortex and spin down reagents. If using *96 reaction kit*, combine **2.8 µl NEBNext ARTIC Human Control Primer Pairs 2** with

168 µl NEBNext ARTIC SARS-CoV-2 Primer Mix 2 . Use **1.75 µl combined mix** for each Pool Set B reaction.

A	B
COMPONENT	VOLUME
cDNA (Step 5)	4.5 µl
(lilac) Q5 Hot Start High-Fidelity 2X MM	6.25 µl
NEBNext ARTIC SARS-CoV-2 Primer Mix 2*	1.75 µl
Total Volume	12.5 µl

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

Flick the tube or pipet up and down 10 times to mix followed by a quick spin.

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8

9

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.
** It is very important to set up the annealing and extension temperature to 63°C.

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

Cleanup of cDNA Amplicons

10 **If you prefer to omit the cleanup step, please use the "**Express Protocol without PCR Bead Cleanup" for E7660 on protocols.io. Otherwise proceed with step 11.

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.

11 For each sample, combine pool A and pool B PCR Reactions.

12 Vortex SPRIselect or NEBNext Sample Purification Beads to resuspend.

13

Add **20 µl (0.8X) resuspended beads** to the combined PCR reaction. Mix well by flicking the tube or pipetting up and down 10 times to mix and a very short 2-3 seconds quick centrifugation. Be sure to stop the centrifugation before the beads start to settle out.

14 10m

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Incubate samples at **Room temperature** for **00:10:00** .

15 Place the tubes on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample **00:00:01** to collect the liquid from the sides of the tube before placing on the magnetic stand. 1s

16 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.

17  30s

Add **500 µl 80% freshly prepared ethanol** to the tube 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.

18  31s

Repeat previous step once for a total of two washes:
Add **500 µl 80% freshly prepared ethanol** to the tube 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 for 00:00:01 , place back on the magnetic stand and remove traces of ethanol with a p10 pipette tip.

19 Air dry the beads for **00:00:30** while the tube is on the magnetic stand with the lid open. 30s

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.

20 

Remove the tube from the magnetic stand. Elute the DNA target from the beads by adding **21 µl Nuclease-free water** .

21  10m

Mix well by flicking the tube or pipetting up and down 10 times to mix and followed by a very short centrifugation. Incubate for **00:10:00** at **Room temperature** . If necessary, quickly spin the sample to collect the liquid from

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the sides of the tube or plate wells before placing back on the magnetic stand.

22

Place the tube on the magnetic stand. After 2 minutes (or when the solution is clear), transfer **20 µl** to clean PCR tubes.

23

Assess the concentration of the DNA targets. We recommend using a Qubit fluorometer for concentration assessment. Use 1 µl of sample for the Qubit fluorometer. Amplicons may also be run on a Bioanalyzer® or a TapeStation to confirm 400 bp size of amplicons.

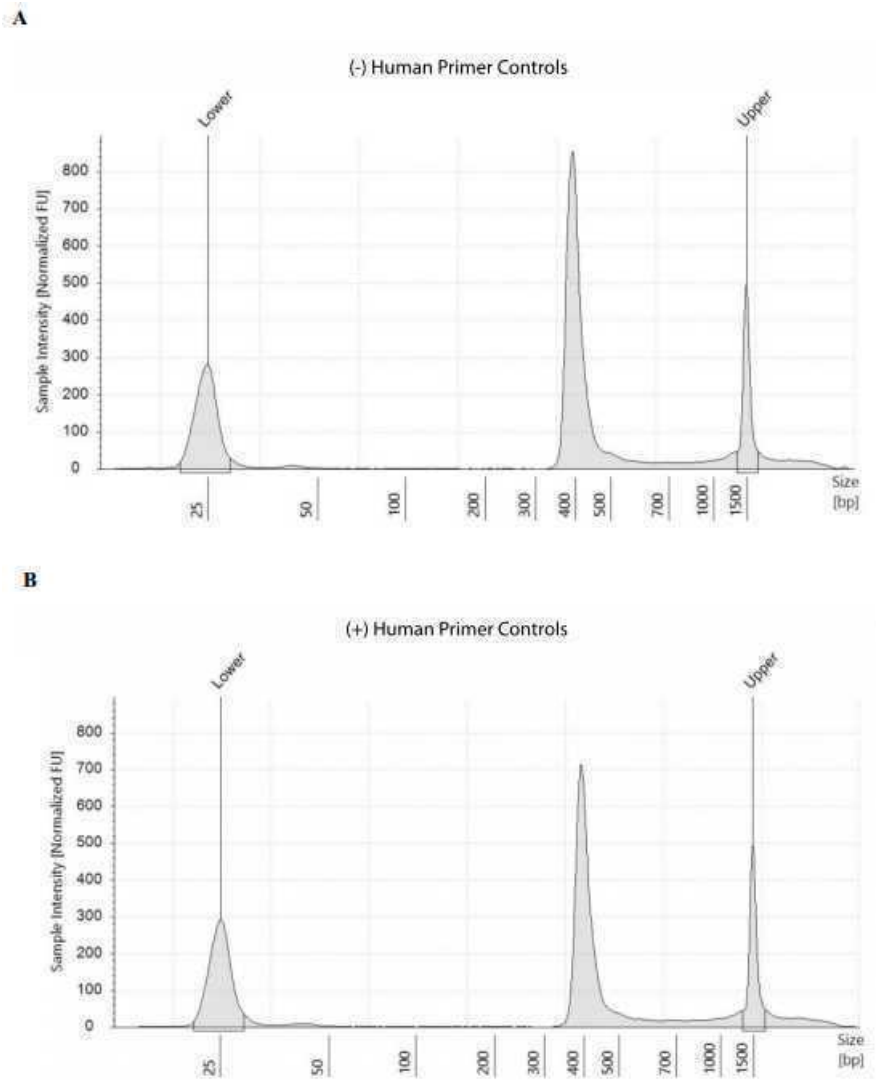

Figure 24: Example of cDNA amplicons generated from 1000 genome copies of SARS CoV-2 in the absence (A) and presence (B) of human primer controls.

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

NEBNext End Prep

24

Use the Qubit readings from Step 23 to dilute 50 ng of the Targeted cDNA Amplicons sample with nuclease-free water to a final volume of 12.5 µl (4 ng/µl). Add the following components to a PCR tube (End Prep Reaction and Buffer can be pre-mixed and stable 🌡 **On ice** for 4 hours):

A	B
COMPONENT	VOLUME
Targeted cDNA Amplicons (Step 22)	12.5 µl
(green) NEBNext Ultra II End Prep Reaction Buffer	1.75 µl
(green) NEBNext Ultra II End Prep Enzyme Mix	0.75 µl
Total Volume	15 µl

25

Flick the tube or pipet up and down 10 times to mix the solution. 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.

26

20m

Place in a thermocycler, with the heated lid set to ≥ 75°C, and run the following program:

00:10:00 @ 🌡 **20 °C**

00:10:00 @ 🌡 **65 °C**

Hold at 🌡 **4 °C**

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

Barcode Ligation

27

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

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A	B
COMPONENT	VOLUME
(white) Nuclease-free water	6 µl
End-prepped DNA (Previous Step)	1.5 µl
Native Barcode*	2.5 µl
(red) Blunt/TA Ligase Master Mix**	10 µl
Total Volume	20 µl

* Native Barcodes are provided in Oxford Nanopore Technologies Native Barcoding Expansion 1-12 (EXP-NBD104) and 13-24 (EXP-NBD114)
** Mix the Blunt/TA Ligase Master Mix by pipetting up and down several times prior to adding to the reaction

28 

Flick the tube or pipet up and down 10 times 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.

29 

31m

Incubate at **Room temperature** for **00:20:00**

Incubate at **65 °C** for **00:10:00** .

Place **On ice** for **00:01:00** .

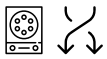
30 Pool all barcoded samples into one 1.5 ml DNA LoBind Tube.

Cleanup of Barcoded DNA

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

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.

32 Vortex NEBNext Sample Purification Beads to resuspend.

33 

1s

Citation: New England Biolabs (05/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore TechnologiesÃÂ®) E7660. https://dx.doi.org/10.17504/protocols.io.budxns7n

Add 0.4X resuspended beads to pooled, barcoded samples (Step 30), for example, if you are pooling 24 libraries (which amounts to 480 µl total), add **192 µl of resuspended Sample Purification beads** to the 480 µl of pooled sample. Flick the tube or pipet up and down 10 times to mix to resuspend pellet. Perform a quick spin for **00:00:01** to collect all liquid from the sides of the tube.

34 

10m

Incubate samples on bench top for **00:10:00** at **Room temperature** .

35 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.

36 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.

37 

1s

Wash the beads by adding **250 µl Short Fragment buffer (SFB)** . Flick the tube or pipet up and down to mix to resuspend pellet. If necessary, quickly spin the sample for **00:00:01** to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

38 Place the tube on an appropriate magnetic stand for 2 minutes (or until the solution is clear) to separate the beads from the supernatant. Remove the supernatant.

39 

3s

Repeat previous 2 steps once for a total of two washes:
Wash the beads by adding **250 µl Short Fragment buffer (SFB)** . Flick the tube or pipet up and down to mix to resuspend pellet. If necessary, quickly spin the sample for **00:00:03** 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 for 2 minutes (or when the solution is clear) to separate the beads from the supernatant. Remove the supernatant.

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

40 Add **200 µl 80% freshly prepared ethanol** to the tube while on 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.

30s

41 A quick spin and place the sample tube on the magnetic stand, remove any residual ethanol.

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42 Air dry the beads for **00:00:30** while the tube is on the magnetic stand with the lid open. 30s

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.

43 

Remove the tube from the magnetic stand. Elute the DNA target from the beads by adding **33 µl Nuclease-free water** .

44  1s

Resuspend the pellet by flicking the tube or pipetting up 10 times and down to mix. Incubate for at least 2 minutes at **Room temperature** . If necessary, quickly spin the sample for **00:00:01** to collect the liquid from the sides of the tube before placing back on the magnetic stand.

45 

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 or PCR tube.

46 

We recommend assessing cDNA concentrations with a Qubit fluorometer. Use 1 µl for the Qubit fluorometer.

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

Adapter Ligation

47 

Use the Qubit readings from Step 46 to dilute 60 ng of the Native barcoded DNA pool with nuclease-free water to a final volume of 30 µl (2 ng/µl). Add the following components into a 1.5 ml Eppendorf DNA LoBind Tube or nuclease-free PCR tube:

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A	B
COMPONENT	VOLUME
Native barcoded and purified DNA (Step 45)	30 µl
Adapter Mix II (AMII)**	5 µl
(red) NEBNext Quick Ligation Reaction Buffer *	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.

48  1s

Flick the tube to mix solution. Perform a quick spin for **00:00:01** 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.

49  20m

Incubate at **25 °C** or at **Room temperature** for **00:20:00** .

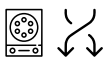
50 Proceed to Cleanup of Adapter-ligated DNA in the next section.

Cleanup of Adapter Ligated DNA 20m 3s

51

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.

52 Vortex NEBNext Sample Purification Beads to resuspend.

53  1s

Add **50 µl (1X) resuspended beads** to the ligation mix. Mix well by flicking the tube to mix followed by a quick spin for **00:00:01** .

Citation: New England Biolabs (05/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore TechnologiesÃÂ®) E7660. https://dx.doi.org/10.17504/protocols.io.budxns7n

54  10m

Incubate samples for **00:10:00** at **Room temperature** .

55 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.

56 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.

57 

Wash the beads by adding **250 µl Short Fragment Buffer (SFB)** . Flick the tube 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.

58 Wait for 2 minutes (or until the solution is clear) to separate the beads from the supernatant. Remove the supernatant.

59 

Repeat previous 2 steps once for a total of two washes:
Wash the beads by adding **250 µl Short Fragment Buffer (SFB)** . Flick the tube 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.
Wait for 2 minutes (or when the solution is clear) to separate the beads from the supernatant. Remove the supernatant.

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.

60 

Remove the tube from the magnetic stand. Elute the DNA target from the beads by adding **15 µl Elution Buffer (EB)** provided in SQK-LSK109 kit from Oxford Nanopore.

61  10m

Resuspend the pellet well in EB buffer by flicking the tube. Incubate for **00:10:00** 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.

62 

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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.

63

Use Qubit to quantify **1 µl DNA sample** . Follow Oxford Nanopore Protocol SQK-LSK109 to prepare MinION® flow cell and DNA library sequencing mix using up to 20 ng adapter-ligated cDNA sample (previous step).

Note: After normalizing the DNA to 20 ng, if the volume is less than 12 µl, then top off the sample volume to 12 µl with EB.

Citation: New England Biolabs (05/25/2021). NEBNextÃÂ® ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore TechnologiesÃÂ®) E7660. https://dx.doi.org/10.17504/protocols.io.budxns7n