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ARTIC HELP protocol for amplicon-based viral genome sequencing

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We use this protocol and it's working

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Abstract

ARTIC HELP (**H**omebrew **E**nzymes for **L**ibrary **P**reparation) is a protocol for amplicon-based viral genome sequencing, developed by systematically testing enzyme and buffer alternatives to the standard reagents used in the ARTIC LoCost protocol and the Oxford Nanopore Technologies (ONT) native barcoding workflow. This includes substitutions for RT, PCR, end-prep, barcode ligation, and adapter ligation steps. Rather than attempting to replace all commercial enzyme mixes, our goal was to provide practical, accessible substitutions using reagents commonly stocked in standard molecular biology labs. Each step in the HELP workflow is modular and compatible with the LoCost protocol, allowing users to substitute individual components based on availability, or local reagent access.
We validated the protocol using clinical samples of SARS-CoV-2 and Norovirus GII, confirming its reliable performance under real-world conditions. The ARTIC HELP offers a robust and cost-effective solution that helps overcome supply chain disruptions, maintain sequencing continuity, and supports pathogen genomic surveillance efforts.
NOTE: You may use your own RT-PCR protocol to generate amplicons, then proceed with the HELP library preparation steps (end-prep, barcode ligation, and adapter ligation). Adjust the input molar concentration based on amplicon size, ONT recommends 200 fmol per sample for optimal ligation with the Native Barcoding Kit.

Please cite the associated publication when using or adapting this workflow.

Materials

	A	B	C
	Reagent	**Supplier**	**Catalog #**
	M-MLV Reverse Transcriptase	Promega	M1705
	RNaseOUT Recombinant Ribonuclease Inhibitor	Invitrogen	10777019
	Random Hexamers (50 µM)	Invitrogen	N8080127
	dNTP Solution Mix (10mM each)	Thermo Fisher	R0192
	Nuclease-Free Water (150 ml)	Promega	P1195
	Q5 Hot Start High-Fidelity DNA Polymerase	NEB	M0493
	Platinum SuperFi DNA Polymerase	Invitrogen	12351010
	High Fidelity PCR EcoDry Premix	TAKARA	639280
	PCR Clean - DX (SPRI magnetic Beads)	Line Biosciences	C-1003-50
	Qubit 1X dsDNA HS Assay Kit-500 assays	Invitrogen	Q33231
	T4 Polynucleotide Kinase (10 U/µL)	NEB	M0201S
	T4 DNA Polymerase (3 U/µL)	NEB	M0203S
	Taq DNA Polymerase, recombinant (5 U/uL)	Thermo Scientific	EP0401
	10X T4 DNA Ligase Reaction Buffer	NEB	B0202S
	Polyethylene glycol solution 40 % (w/w) in H2O, average mol wt 8000	Sigma-Aldrich	P1458-25ML
	T4 DNA Ligase (2,000,000 U/ml)	NEB	M0202M
	Native Barcoding Kit 96 V14	ONT	EXP-NBD196
	Flow cells R10.4.1	ONT	FLO-MIN114

Troubleshooting

Before start

- Ensure proper sample collection and RNA extraction.
- Use at least 12 samples (including negative control) for optimal sequencing yield and barcode performance.
- Prepare master mixes with 10% extra volume to account for pipetting variation.
- Work in three separated zones: clean zone, pre-PCR and post-PCR zones.
- Clean surfaces with 10% bleach, followed by 80% ethanol to remove bleach residue. Avoid using bleach on metal surfaces. Use UV as a final decontamination step.
- Use dedicated pipettes and equipment for each zone.
- Include a negative control during RT (using NFW instead of RNA) and carry it through the full workflow.

I. RT (cDNA preparation)

1h 10m

1 Mix the following components in PCR strip tubes or a 96-well plate. Gently mix by pipetting, then briefly spin down to collect the liquid at the bottom.

	A	B
	Component	**Volume (µL) per rxn**
	Random hexamers 50µM	1
	dNTP (10mM each)	1
	Template RNA	12
	Total	**14 µL**

2 Incubate at 65 °C for 00:05:00 nap cool on ice.

5m

3 Make up the following master mix and add 6 µL to the 14 µL annealed RNA:

	A	B
	Component	**Volume (µL) per rxn**
	5X M-MLV Buffer	4
	RNase OUT	1
	M-MLV (200 U/µL)	1
	Total	**6 µL**

4 Set-up the following incubation using PCR thermocycler:
25 °C for 00:05:00

1h 5m

🌡 42 °C for ⏲ 00:50:00
🌡 70 °C for ⏲ 00:10:00
🌡 4 °C Hold

II. Multiplex PCR

5m 45s

5 Primer pool preparation

Note

Primer pool preparation for SARS-CoV-2 is described in the *ARTIC SARS-CoV-2 sequencing protocol v4 (LSK114)* [Version 4], available at: https://dx.doi.org/10.17504/protocols.io.bp2l6n26rgqe/v4.

The primer scheme used in this protocol is *artic-sars-cov2/400/v4.1.0*, available at: **https://labs.primalscheme.com/detail/artic-sars-cov-2/400/v4.1.0/**

If you are using a different primer scheme for another virus, prepare the primer pools accordingly. Primers should be used at a final concentration of **15 nM per primer**. For guidance, see:
Quick, J. *et al.* (2017) Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nature Protocols*, 12, 1261–1276. https://doi.org/10.1038/nprot.2017.066

6 Depending on the polymerase used (**Platinum, EcoDry or Q5**), set up two PCR reactions per sample (**Pool 1** and **Pool 2**) in strip-tubes or 96-well plates. Gently mix by pipetting, and briefly spin down to collect liquid at the bottom. For plates use plate spinner centrifuge.

STEP CASE

Platinum™ SuperFi DNA Polymerase (Invitrogen)

52 steps

	A	B
	Component	**Volume (µL) per rxn**
	5X SuperFi Buffer	5
	10mM dNTP	0.5
	Primer Pool 1 or Pool 2 (10µM) v4.1	4

	A	B
	Platinum SuperFi DNA Pol (2 U/µL)	0.25
	Nuclease-free water	12.75
	Total	**22.5 uL**

7

Note

Continue with **steps 8 and 9** in the *Multiplex PCR* section under the **“Platinum” Step Case**, then follow **Sections III to X** of the protocol using the same **“Platinum” Step Case**. These steps are the same for all polymerases.

8 Add 2.5 µL cDNA to each of the PCR reactions (Pool 1 & Pool2), now the total reaction volume 25 µL . Gently mix by pipetting and pulse spin the tube to collect liquid at the bottom.

9 Start the following program on the PCR thermocycler:

5m 45s

Step	**Temperature**	**Time**	**Cycles**
Heat Activation	98 °C	00:00:30	1
Denaturation	95 °C	00:00:15	30
Annealing/Extension	63 °C	00:05:00	
Hold	4 °C		

III. Amplicon Dilution and Pooling

10 Label strip-tubes/plate and combine the following volumes of each PCR reaction with NFW to get 50 µL total volume:

40 µL NFW
\+ 5 µL Pool 1 PCR amplicon
\+ 5 µL Pool 2 PCR amplicon

IV. Post-PCR Amplicon Clean Up & Quantification

26m 30s

11 Before starting, prepare fresh 80% ethanol and nuclease-free water (NFW).

Note

You can perform post-PCR amplicon clean-up in different formats:

- *In **1.5 mL Eppendorf tubes** (recommended: Invitrogen DynaMag-2 Magnet; cat# 12321D)*
- *In **96-well plates** for large-scale clean-up (recommended: Permagen 96-Well Side Pull Bar Magnet PCR Separation Plate; cat# MSP750KT)*
- *In **0.2 mL strip tubes** for small-scale clean-up (recommended: NEBNext Magnetic Separation Rack; cat# S1515S)*

Be especially careful with contamination when working with 96-well plates!

- Use multichannel pipettes when working with 96-well plates or strip tubes to ensure consistent handling and avoid sample mix-ups.
- Use plastic reagent reservoirs when performing clean-up in 96-well plates or strip tubes.
- Always prepare aliquots of magnetic beads in advance to minimize contamination risk.
- Vortex magnetic beads thoroughly before use to ensure full resuspension. The bead solution should appear as a uniform brown suspension.
- Always prepare fresh 80% ethanol before starting. During ethanol washes, add the ethanol slowly along the side of the well or tube, on the side opposite to the pellet.

12 Add 50 µL of magnetic beads (1× ratio) to each well/tube. Mix gently by pipetting (or by flicking, if using 1.5 mL tubes). Briefly spin down and incubate for 00:05:00 at Room temperature . 5m

13 Place the tubes or plate on the appropriate magnetic rack or plate. Incubate for 00:05:00 or until beads are fully pelleted and the supernatant is clear. 5m

14 Carefully remove and discard the supernatant without disturbing the pellet.

15 Repeat the ethanol wash step: 1m

- *For **1.5 mL tubes***: After the second wash, spin briefly, return to magnet, remove residual ethanol using a p10 pipette, and air dry the pellet for 00:01:00 (or until the shine disappears but not to the point of cracking).
- *For **96-well plates or strip tubes***: After the second ethanol addition, remove 30 µL , briefly spin (using a plate centrifuge for plates), then carefully remove all residual ethanol. Do not overdry.

16 Resuspend the pellet: 10m

*In **1.5 mL tubes***: Remove from magnet and resuspend in 35 µL of NFW by flicking. Briefly spin down and ensure that the beads not pelleted at the bottom. Incubate for

00:05:00 at Room temperature .

*In **96-well plates or strip tubes***: Remove from magnet, resuspend in 35 µL of NFW by gentle pipetting (~10x). Briefly spin down and ensure that the beads not pelleted at the bottom. Incubate for 00:05:00 at Room temperature .

17 Place the tubes or plate back on the magnet. Allow the beads to pellet for 00:05:00 , or until the eluate is clear. 5m

18 Carefully transfer 35 µL of the eluate to a new **1.5 mL LoBind tube, or 96-well plate, or strip-tubes**. Avoid transferring beads. If you notice beads in the tip, elute into a slightly smaller volume (e.g., 32 µL) to ensure purity.

19 Quantify samples and the negative control using a fluorometer such as a Qubit or Quatus.

Note

Samples should be **≥5 ng/µL** for optimal sequencing performance.
The negative control should yield no detectable signal.

You may store samples at 4 °C overnight, or freeze at -20 °C for long-term storage prior to sequencing.

V. Amplicon End Prep

1h

20 Place enzymes on ice. Do not vortex the enzymes. Thaw T4 DNA Ligase Reaction Buffer at room temperature and then, place on ice. Always spin down tubes before opening.

Note

The ATP in the T4 DNA Ligase Reaction Buffer is essential for the ligation reaction but can be broken down by repeated freeze-thaw cycles. To avoid this, aliquot the ligase buffer from each new stock of DNA ligase and keep at -20 °C . Make the aliquots small enough for single-use and make sure to completely defrost and mix well before you aliquot.

21 For each sample, prepare the following mix and aliquot 6.7 µL per sample in strip-tubes or 96-well plate. Then add 3.3 µL of amplicons from the previous section.

A	B
Component	**Volume (µL) per rxn**
T4 PNK (10U/µL)	0.2
T4 Pol (3U/µL)	0.07
Taq Pol (5U/µL) Invitrogen	0.08
10x T4 Ligase Reaction Buffer (with 10mM ATP)	1
dNTP (10mM)	0.5
PEG 8000 (40%)	1.25
Nuclease-Free Water	3.6
amplicons (from previous step)	3.3
Total	**10 µL**

Note

Caution: PEG 8000 is highly viscous.
Pipette slowly and carefully to ensure accurate volumes and avoid bubbles.

22 Set-up the following incubation using PCR thermocycler: 1h

20 °C for 00:30:00
65 °C for 00:30:00
4 °C Hold

Note

For best results EP products shouldn't be frozen before barcoding. For best results proceed directly to barcode ligation.

VI. Barcode Ligation

40m

23 repare ONT native barcodes.

Note

ONT native barcodes can be added directly to the reaction (1.25 µL /reaction) or used in diluted form (3 µL /reaction), see dilution instruction below. Keep in mind that the volume of NFW per reaction will vary depending on which barcode format is used. To maintain a consistent final reaction volume and barcode concentration:

- When using diluted barcodes, add 1.5 µL of NFW per reaction.
- When using undiluted barcodes, add 3.25 µL of NFW per reaction.

Barcode Dilution Instructions:
To streamline the workflow, native barcodes can be pre-diluted and aliquoted into strip tubes or 96-well plates in advance (e.g., 3 µL per well or tube).

- Dilute each barcode at a 1.4:1 NFW-to-barcode ratio.
  For example: add 11.2 µL of NFW to 8 µL of native barcode (as provided by ONT in 96-well plates).
- Dispense 3 µL aliquots of each diluted barcode into new 96-well plates or strip tubes.
- Store diluted barcode plates or strips at −20°C.

You can organize barcode plates or tubes based on anticipated library size. For example:

- Half-plates containing barcodes NB01–56 and NB57–96
- Strip tubes divided into groups such as NB01–24, NB25–48, etc.

24 Mix all components in a new PCR strip tube or 96-well plate for each sample. Gently pipette to mix and briefly spin down to collect the liquid at the bottom.

A	B	C
Component	**ONT Barcode (µL/rxn)**	**Prediluted ONT Barcode (µL/rxn)**
NBXX Barcode	1.25	3
EndPreped amplicons	1.5	1.5
10x T4 Ligase Reaction Buffer	1	1
PEG8000 (40% w/w)	2.5	2.5

A	B	C
T4 Ligase (2000U/µL)	0.5	0.5
Nuclease-Free Water	3.25	1.5
Total	**10 µL**	**10 µL**

Note

Prepare the ligation mix based on the barcode format you are using:
- If using **undiluted ONT barcodes**, follow the volumes listed under the **"ONT Barcode"** column.
- If using **pre-diluted barcodes**, follow the volumes listed under the **"Prediluted ONT Barcode"** column

Note

Caution: PEG 8000 is highly viscous.
Pipette slowly and carefully to ensure accurate volumes and avoid bubbles.

25 Set-up the following incubation using PCR thermocycler: 40m

20 °C for 00:30:00
70 °C for 00:10:00
4 °C Hold

Note

You can freeze at -20 °C (eg., overnight) the samples at this point if you need to pause the experiment.

VII. Pooling of Barcoded Samples 24m

26 POOL barcoded amplicons in a clean 1.5 mL Eppendorf DNA LoBind tube.

Note

POOL barcoded amplicons in a clean 1.5 mL Eppendorf DNA LoBind tube:
- *If processing <32 samples, pool all* 10 µL *from each barcoding reaction.*
- *If processing 33-47 samples, pool* 8 µL *from each barcoding reaction.*
- *If processing 48-80 samples, pool* 5 µL *from each barcoding reaction.*
- *If processing 81-96 samples, pool* 3 µL *from each barcoding reaction.*

27 Vortex the SPRI magnetic beads to resuspend. Add *0.4×* volume of beads to the pooled reaction and mix by flicking.

Note

To calculate 0.4×: Multiply the total pooled volume by 0.4.
For example, 12 samples × 10 µL = 120 µL → 120 × 0.4 = 48 µL of magnetic beads.

28 Incubate the reaction for 00:05:00 at Room temperature . 5m

29 Place the tube into a magnetic rack for 00:05:00 . 5m

30 Keep on magnet and remove all supernatants.

31 Wash the beads with 250 µL SFB (ONT). Remove a tube from the magnetic rack and fully resuspend the pellet in SFB (gently by either flicking or pipetting). Then return the tube to the magnet and allow the beads to pellet. Allow 00:05:00 contact time with the magnet. 5m

32 Repeat the SFB wash go to step #30 go to step #31

33 Keep on magnet and remove all supernatants. Pulse centrifuge and remove any residual SFB.

Note

There is no need to air dry the beads after SFB washes.

34 Remove all supernatant. Keep tube on the magnet and add 200 µL of 80 % Ethanol to bathe the pellet. Carefully remove and discard ethanol, being careful not to touch the bead pellet.

Note

Only perform 1x 80% ethanol wash

35 Pulse centrifuge to collect all liquid at the bottom of the tube and carefully remove as much residual ethanol as possible using a P10 pipette.

36 With the tube lid open incubate for 00:01:00 or until the pellet loses its shine. 1m

Note

do not allow the pellet dries completely, it will crack and become difficult to resuspend.

37 Add 32 µL NFW water, mix gently by flicking and incubate for 00:05:00 at Room temperature . 5m

38 Place the tube into a magnetic rack for 00:03:00 or until the eluate is clear and colourless. 3m

39 Elute 32 µL sample to a clean 1.5 mL Eppendorf DNA LoBind tube.

Note

Be careful not to take any beads! Use light!

40 Take 1 µL for quantification using a fluorometer such as a Qubit or Quatus.

Note

at least 30 ng total concentration (or 1 ng) is needed for proper yield.

Note

You can store the pooled sample at 4 °C overnight and continue the library preparation the next day, if needed.

VIII. Adapter Ligation

20m

41 Spin down the T4 DNA Ligase (2000U/µL) and place on ice. Do not vortex the T4 Ligase; gently invert the tube to mix. Flick and briefly spin down the ONT Adapter Mix and place on ice.

Note

If you are using the ONT V14 chemistry kit, the tube labeled Native Adapter (NA) corresponds to Adapter Mix II (AMII) in the ONT V9 chemistry kit.

42 Perform the adapter ligation of the pooled and barcoded amplicons. In a clean 1.5 ml Eppendorf LoBind tube, add the following reagents:

	A	B
	Component	**Volume (µL)**
	Pooled barcoded sample	30
	Adapter Mix ONT	5
	10x T4 Ligase Reaction Buffer	14
	PEG8000 (40% w/w)	17.5

A	B
T4 Ligase (2000U/µL)	1
Nuclease-Free Water	2.5
Total	**70 µL**

Note

Caution: PEG 8000 is highly viscous.
Pipette slowly and carefully to ensure accurate volumes and avoid bubbles.

43 Ensure the components are thoroughly mixed by gentle flicking, and briefly spin down.

44 Incubate the reaction for 00:20:00 at Room temperature (or if using PCR thermocycler: 20°C for 20 minutes and then 4°C Hold). 20m

IX. Final Clean Up

22m

45

Note

BEFORE TO START:

- Allow Flow cell to equilibrate to room temperature for 30 min. During the final clean up, the flow cell can be QC'd.
- **Do NOT use Ethanol!!!** for the final clean-up step as it would denature motor proteins on adaptors. SFB will remove excess adapter without damaging the adapter-protein complexes.
- Vortex magnetic beads thoroughly before use to ensure they are well resuspended; the solution should be a homogenous brown colour.

46 Add 70 µL magnetic beads (1× ratio), and mix gently by flicking the tube. Pulse centrifuge to collect all liquid at the bottom of the tube.

47 Incubate for 00:05:00 at Room temperature . 5m

48 Place the tube into a magnetic rack for 00:05:00 5m

49 Remove all supernatants and wash beads with 250 µL SFB (ONT). Resuspend beads in SFB completely by pipette mixing/flicking. Pulse centrifuge to collect all liquid at the bottom of the tube.

50 Return on the magnet and allow the beads to pellet. Allow 00:05:00 contact time with the magnet. 5m

51 Repeat the SFB wash step twice. go to step #49 go to step #50

52 Remove all supernatant. Then spin down and carefully remove any residual SFB.

Note

You do not need to allow to air dry with SFB washes.

53 Add 15 µL of EB (ONT) and gently resuspend by flicking. Incubate 00:05:00 a Room temperature 5m

54 Place the tube into the magnetic rack for 00:02:00 or until the eluate is clear and colourless. 2m

55 Transfer 15 µL of final library to a new 1.5mL Eppendorf DNA LoBind tube.

Note

Be careful not to take any beads!

56 Take 1 µL for quantification using a fluorometer such as a Qubit or Quatus.

Note

at least 15 ng total concentration (or 1 ng/µL) is needed for proper yield.

Note

The final library can be stored at 🌡 4 °C for up to one week, if necessary. However, it is recommended to proceed to sequencing as soon as possible for best results.

MinION Sequencing

57 Prime and load the flow cell with the final library, following the current ONT protocol for the Native Barcoding Kit you are using.

Note

For detailed instructions on **priming and loading a MinION flow cell**, see this video: https://youtu.be/Pt-iaemrM88?si=5PjpFCTWlMEkFGrb

58 Start the sequencing run using MinKNOW.

Note

If using live basecalling, make sure to enable “Barcode both ends” in the basecalling settings.
We also recommend using High Accuracy basecalling mode and enabling the “Trim barcodes” option in the barcoding settings.

HAPPY SEQUENCING 🚀