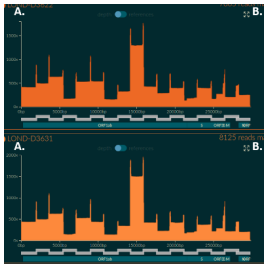
Version 1

Long reads nanopore sequencing to recover SARS-CoV-2 whole genome V.1



DOI
https://dx.doi.org/10.17504/protocols.io.bfe4jjgw

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Protocol Citation: Paola Resende . Long reads nanopore sequencing to recover SARS-CoV-2 whole genome . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bfe4jjgw

Created: April 22, 2020

Last Modified: April 30, 2020

Protocol Integer ID: 36028

Keywords: nanopore, long reads, sequencing, genome, SARS-CoV-2 sequencing, RSV sequencing, Parainfluenza sequencing, pathogen genomic unit, facilities at pathogen genomic unit, sequencing facility, ucl genomic, respiratory virus, whole genome, pathogen, nanopore, amplicon tiling strategy, long read, amplicon library,

Abstract

This protocol describes step-by-step instructions for building long (~2kb) amplicon libraries to recover SARS-CoV-2 genomes using the nanopore sequencing.

It can be applied for sequencing on the MinION or GridION.

This protocol was developed in collaboration with the Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz Institute, FIOCRUZ, Brazil and the two sequencing facilities at Pathogen Genomic Unit (PGU) and UCL Genomics, University College London (UCL), United Kingdom.

This document describes the manual steps to perform the protocol, but for further information about the automation of the protocol, please, contact PGU-UCL.
Manager: Rachel Williams
https://www.ucl.ac.uk/infection-immunity/pathogen-genomics-unit

This protocol is based in the amplicon tiling strategy described previously by Quick J et al 2017. However, we have applied this strategy to recover long reads (2kb), then some adjustments were performed in the protocol.

Citation

Quick J, Grubaugh ND, Pullan ST, Claro IM, Smith AD, Gangavarapu K, Oliveira G, Robles-Sikisaka R, Rogers TF, Beutler NA, Burton DR, Lewis-Ximenez LL, de Jesus JG, Giovanetti M, Hill SC, Black A, Bedford T, Carroll MW, Nunes M, Alcantara LC Jr, Sabino EC, Baylis SA, Faria NR, Loose M, Simpson JT, Pybus OG, Andersen KG, Loman NJ (2017)
. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples.
Nature protocols.

https://doi.org/10.1038/nprot.2017.066 LINK

Materials

MATERIALS

- NEBNext Ultra II End Repair/dA-Tailing Module - 96 rxns **New England Biolabs** **Catalog #**E7546L
- NEBNext Ultra II Ligation Module - 96 rxns **New England Biolabs** **Catalog #**E7595L
- Qubit® dsDNA HS Assay kit **Thermo Fisher Scientific** **Catalog #**Q32854
- NEB Blunt/TA Ligase Master Mix **Catalog #**M0367
- Agencourt AMPure XP beads **Beckman Coulter** **Catalog #**A63881
- SuperScript™ IV First-Strand Synthesis System **Thermo Fisher** **Catalog #**18091200
- ONT MinION Flow Cell R9.4.1 **Oxford Nanopore Technologies** **Catalog #**FLO-MIN106D
- Ligation sequencing kit 1D **Oxford Nanopore Technologies** **Catalog #**SQK-LSK109
- Flowcell Wash Kit **Oxford Nanopore Technologies** **Catalog #**EXP-WSH003
- NEB Q5® Hot Start High-Fidelity 2X Master Mix **New England Biolabs** **Catalog #**M0494L
- Native Barcoding Expansion 1-12 (PCR-free) **Oxford Nanopore Technologies** **Catalog #**EXP-NBD104
- Native Barcoding Expansion 13-24 (PCR-free) **Oxford Nanopore Technologies** **Catalog #**EXP-NBD114
- Primer set SARS-CoV-2_v1

RT (Optional)

- SuperScript™ IV First-Strand Synthesis System. (200 reactions) Cat: 18091200 Invitrogen

PCR (Optional)

- Q5® Hot Start High-Fidelity 2X Master Mix Cat: M0494
- Primer set SARS-CoV-2_v1 Pool 1 and Pool 2 (2kb) Paola Resende

Clean-up and QC

- Qubit ds DNA HS kit. Cat: Q32854 Thermofisher
- Ampure XP. Cat: A63881 Beckman Coulter

End-Prep, Barcoding, Adapter Ligation and Sequencing

- NEBNext® Ultra™ II Ligation Module. Cat: E7595L NEB
- NEBNext® Ultra™ II End Repair/dA-Tailing Module. Cat: E7546L NEB
- NEB Blunt/TA Ligase Master Mix. Cat: M0287L NEB
- Ligation Sequencing Kit. Cat: SQK-LSK109 ONT
- Native Barcoding Expansion 1-12 (PCR-free). Cat: EXP-NBD104 ONT
- Native Barcoding Expansion 13-24 (PCR-free). Cat: EXP-NBD114 ONT
- Flow Cell (R9.4.1) ONT

Flow cell wash

- Flow Cell Wash Kit. Cat: EXP-WSH003 ONT

Real-time data analysis

- Computer operational system LINUX

- MinKnow
- RAMPART (https://github.com/artic-network/rampart)

Primers dilution

1 **Instructions for Primer Scheme dilution:**

Note

The primer scheme dilution should be prepared in **Master Mix Hood** or **Clean Room**.
To avoid cross-contamination make sure that your original stock reagents have no contact with RNA or any amplified DNA material.

If lyophilised each primer should be resuspended to [M] 100 micromolar (µM) **(Stock dilution 1)**

The primers from **Primer Scheme 2kb_v1 Pool 1** and **Primer Scheme 2kb_v1 Pool 2** are:

Primer Scheme 2kb_v1 Pool 1: F1, R1, F3, R3, F5, R5, F7, R7, F9, R9, F11, R11, F13, R13, F15, R15, F17, R17
(9 primers pairs)

Primer Scheme 2kb_v1 Pool 2: F2, R2, F4, R4, F6, R6, F8, R8, F10, R10, F12, R12, F14, R14, F16, R16
(8 primers pairs)

		Primer Scheme 2kb_v1 Pool 1		**Primer Scheme 2kb_v1 Pool 2**
	Oligo name	**Oligo sequence (5' to 3')**	**Oligo name**	**Oligo sequence (5' to 3')**
	hCoV_F1_2kb	ACCAACCAACTTTCGATCTCTTGT	hCoV_F2_2kb	CTGCTCAAAATTCTGTGCGTGT
	hCoV_R1_2kb	ACACCACCTGTAATGTAGGCCA	hCoV_R2_2kb	GGTCAGCACCAAAAATACCAGCT
	hCoV_F3_2kb	AGCGGACACAATCTTGCTAAACA	hCoV_F4_2kb	TTGTGCACTTATCTTAGCCTACTGT
	hCoV_R3_2kb	GGTTGTCTGCTGTTGTCCACAA	hCoV_R4_2kb	TGCCAAAAACCACTCTGCAACT

	hCoV_F5_2kb	CACTATTGCAACCTACTGTACTGGT	hCoV_F6_2kb	GTACACTGACTTTGCAACATCAGC
	hCoV_R5_2kb	CGTGTGTCAGGGCGTAAACTTT	hCoV_R6_2kb	AACGGCAATTCCAGTTTGAGCA
	hCoV_F7_2kb	TGTACGCTGCTGTTATAAATGGAGA	hCoV_F8_2kb	TGGTACAACATTTACTTATGCATCAGC
	hCoV_R7_2kb	TTTGACAGCAGAATTGGCCCTT	hCoV_R8_2kb	TGGGTGGTATGTCTGATCCCAA
	hCoV_F9_2kb	CCTTGACCAGGGCTTTAACTGC	hCoV_F10_2kb	AGCAAAATGTTGGACTGAGACTGA
	hCoV_R9_2kb	ATCATCTACAAAACAGCCGGCC	hCoV_R10_2kb	CCAAGCAGGGTTACGTGTAAGG
	hCoV_F11_2kb	GCTGAAATTGTTGACACTGTGAGT	hCoV_F12_2kb	TGCATTCCACACACCAGCTTTT
	hCoV_R11_2kb	AGCACCACCTAAATTGCAACGT	hCoV_R12_2kb	TAACAAAGGCTGTCCACCATGC
	hCoV_F13_2kb	ACAAAAGAAAATGACTCTAAAGAGGGTTT	hCoV_F14_2kb	CAGGCTGCGTTATAGCTTGGAA
	hCoV_R13_2kb	TGTGCTACCGGCCTGATAGATT	hCoV_R14_2kb	CATGACAAATGGCAGGAGCAGT
	hCoV_F15_2kb	TCAGAGTGTGTACTTGGACAATCAA	hCoV_F16_2kb	ACGTGAGTCTTGTAAAACCTTCTTTTT
	hCoV_R15_2kb	GTACCGTTGGAATCTGCCATGG	hCoV_R16_2kb	ACTGCCAGTTGAATCTGAGGGT
	hCoV_F17_2kb	GGAATCATCACAACTGTAGCTGCA		
	hCoV_R17_2kb	TAGGCAGCTCTCCCTAGCATTG		

Primer scheme to recover 2 kilobases amplicon of SARS-CoV-2 genome.

1.1 Prepare the **Primer Scheme 2kb_v1 Pool 1 and Pool 2** [M] 100 micromolar (µM) (stock dilution 2)

Add 20 µL of each primer [M] 100 micromolar (µM) (stock dilution 1) to a 1.5mL tube labelled as **Primer Scheme 2kb_v1 Pool 1**. The final volume will be 360 µL

Add 20 µL of each primer [M] 100 micromolar (µM) (stock dilution 1) to a 1.5mL tube labelled as **Primer Scheme 2kb_v1 Pool 2.** The final volume will be 320 µL

1.2 Prepare the **Primer Scheme 2kb_v1 Pool 1 and Pool 2** [M] 10 micromolar (µM) (concentration to be used)

Add 20 µL of each primer [M] 100 micromolar (µM) of **Primer Scheme 2kb_v1 Pool 1** (stock dilution 2) to a 1.5mL tube labelled as **Primer Scheme 2kb_v1 Pool 1** [M] 10 micromolar (µM)
Add 180 µL of water nuclease-free. The final volume will be 200 µL

Add 20 µL of each primer [M] 100 micromolar (µM) of **Primer Scheme 2kb_v1 Pool 2** (stock dilution 2) to a 1.5mL tube labelled as **Primer Scheme 2kb_v1 Pool 2** [M] 10 micromolar (µM)
Add 180 µL of water nuclease-free**.** The final volume will be 200 µL

Master Mix for cDNA and PCR steps

30m

2 **Instructions to prepare the Maxter Mix for cDNA and PCR steps**

Note

You can save time if you prepare both Master Mix cDNA and PCR to be used on the same day.

Note

The master mix for cDNA and PCR step should be prepared in **Master Mix Hood** or **Clean Room**. To avoid cross-contamination make sure that your original stock reagents have no contact with RNA or any amplified DNA material.

Note

A **Negative Control** (H_2O nuclease free) should be included from cDNA step until the end.

Note

Keep the enzymes on ice and thaw the other reagents at room temperature before placing on ice.

2.1 **Master Mix RT_1:**

Prepare the following components in a 0.2mL 8-strip tube for the number of samples that will be tested (positive samples + a negative control)

VOLUME	COMPONENT
1 µL	50µM random hexamers
1 µL	10mM dNTPs mix (10mM each)

2.2 **Master Mix RT_2:**

Prepare the following components in a 1.5mL tube and keep the Master Mix2-RT on ice. (7uL per sample)

VOLUME	COMPONENT
4 µL	5x SSIV Buffer
1 µL	100mM DTT
1 µL	RNAseOUT RNase Inhibitor
1 µL	SSIV Reverse transcriptase

2.3 **Master Mix PCR Pool 1 and Pool 2:**

Prepare the following components in two 1.5mL tubes and keep the Master Mix PCR Pool 1 and Pool 2 on ice

VOLUME Poo1	VOLUME Pool 2	COMPONENT
5 µL	5 µL	5X Q5 Reaction Buffer

10 µL	10 µL	10 mM dNTPs
0.25 µL	0.25 µL	Q5 Hot Start DNA Polymerase
3.0 µL	3.0 µL	Primer Pool 1 or 2 ([M] 10 micromolar (µM))
4.25 µL	4.25 µL	H_2O Nuclease free

Mix the master mix by inversion several times, briefly spin to collect the contents at the bottom of the tube.
Dispense 22.5 µL per tube 0.2mL 8-strip PCR tubes Pool 1 and Pool 2

Note

Label the side of the tubes, not the lids as the marker may be removed by the heat in the PCR machine.

cDNA

1h 30m

3 **Instructions for the cDNA step:**

Note

This step should be conducted in the pre PCR area.

Note

Keep all the Master Mix (cDNA_2 and PCR) in the fridge.

3.1 Set up the thermocycler for the following condition:

65 °C

3.2 Spin down the **Master Mix RT_1**;

3.3 Add 11 µL RNA to each 0.2 mL tube containing the **Master Mix RT_1**;

3.4 Mix by pipetting; and pulse centrifuge the tubes to collect the contents at the bottom of the tube.

3.5 Incubate the reaction for 65 °C 00:05:00 5m

3.6 Once this step is completed add 7 µL of **Master Mix RT_2** in each tube

3.7 Mix by pipetting and pulse centrifuge the tubes to collect the contents at the bottom of the tube.

3.8 Incubate the reaction in the thermocycler for: 1h
42 °C 00:50:00
70 °C 00:10:00
4 °C hold

Note

The cDNA can be stored in -20 °C

Note

The remaining RNA should be stored in -80 °C

PCR

4 **Instructions for the PCR step:**

Note

This step should be conducted in the pre PCR area.

4.1 Set up the thermocycler for the following conditions:

1 cycle
50 °C 00:10:00
1 cycle
98 °C 00:02:00
35 cycles
98 °C 00:00:10
65 °C 00:05:00
1 cycle
4 °C hold

4.2 Add 2.5 µL cDNA of each sample to each 0.2mL 8-strip PCR tube containing the **Master Mix PCR Pool 1** and **Master Mix PCR Pool 2**

4.3 Mix by pipetting and pulse centrifuge the tubes to collect the contents at the bottom of the tube.

4.4 Incubate in the thermocycler following the conditions described in **substep 4.1**

Clean-up

5 **Instructions for the DNA purification using magnetic beads AMPURE**

Note

This step should be conducted in the PCR room

Note

Keep the Ampure beads and Qubit standards in room temperature 00:15:00 before start.

Note

Prepare fresh [M] 80 % (v/v) ethanol. Do not use [M] 80 % (v/v) ethanol prepared in the previous day.

6 After the RT-PCR, Pool 1 and Pool 2 can be mixed (final volume 50 µL)

7 Add an equal volume of AmpureXP PCR Clean-up beads (50 µL) to the tube (ratio of 1:1 of Ampure beads).

8 Mix gently by either flicking or pipetting 8-10 times.

Note

If long reads (2 Kb) avoid the vortex

9 Incubate for 00:05:00 at room temperature.

10 Pulse centrifuge the tubes to remove any beads or solution from the lid or side of the tube.

11 Place on a magnetic rack and incubate for 00:02:00 or until the beads have pelleted against the magnet and the solution is completely clear.

12 Carefully remove and discard the solution, being careful not to displace the bead pellet.

13 Add 200 µL of fresh room-temperature [M] 80 % (v/v) Ethanol to the pellet.

14 Incubate for 00:01:00 .

15 Carefully remove and discard ethanol, being careful not to displace the bead pellet.

16 Repeat Ethanol wash steps 13-15 to wash the pellet again and continue from step 17.

17 Briefly pulse centrifuge the pellet and carefully remove as much ethanol as possible using a 10 µL tip.

18 Allow the pellet to dry for 00:02:00, being careful not to over-dry (if the pellet is cracking, then it is too dry). Pellet should appear opaque and slightly shiny.

19 Resuspend the pellet thoroughly in 32 µL of water, and incubate for 00:02:00

20 Pulse centrifuge to remove content in the lid.

21 Place on magnet and CAREFULLY remove water and transfer 32 µL to a clean 1.5mL Eppendorf tube.

Note

MAKE SURE that no beads are transferred into this tube. In some cases, a pulse centrifugation can be used to pellet residual beads.

Quality Control (QC)

22 **Instructions to measure the amount of DNA**

Note

Quantify 2 µL of the amplicon library using the Qubit fluorometer following the dsDNA protocol.

Note

To avoid any cross-contamination, we recommend that you remove the total amount of working solution required for your samples and standards from the working solution bottle and then add the required volume to the appropriate tubes instead of pipetting directly from the bottle to each tube.

23 Set up the required number of 0.5mL tubes for standards and samples. The Qubit™ 1X dsDNA HS Assay requires 2 standards.

24 Label the tube lids. Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit™ Fluorometer requires the standards to be inserted into the instrument in the right order.

25 Add the Qubit™ 1X dsDNA 1X buffer to each tube standard tube (190 µL) and each sample tube (198 µL)

26 Add 10 µL of each Qubit™ standards 1 and 2 to the appropriate tube.

27 Add 2 µL of each sample to the appropriate tube

28 Vortex all tubes and incubate at room temperature for 00:02:00 , then proceed to “Read standards and samples”.

29 Carefully record all results to perform the DNA normalisation to prepare the library.

Normalisation

30m

30 **Instructions to normalise the DNA to prepare the library**

For the barcoding step is needed 12.5 µL of DNA in a concentration of 60ng (long reads) per sample.

Note

For an efficient barcoding step, we observe that DNA in the barcoding step should range of 25 to 50 fmol (2kb). Then we are using 60ng per sample.

End-prep and barcoding Master Mix

31 **Instructions to prepare the End-prep and barcoding Master Mix**

Note

The **End-prep and barcoding Master Mix** should be prepared in **Master Mix Hood** or **Clean Room**. To avoid cross-contamination make sure that your original stock reagents have no contact with RNA or any amplified DNA material.

31.1 **Master Mix End-prep:**

Prepare the following components in a 1.5mL tube and keep the Master Mix PCR on ice.

VOLUME	COMPONENT
1.75 µL	Ultra II End Prep Reaction Buffer
0.75 µL	Ultra II End Prep Enzyme Mix

31.2 **Master Mix barcoding:**

Prepare the following components in a 1.5mL tube and keep the Master Mix PCR on ice.

VOLUME	COMPONENT
17.5 µL	Ultra II Ligation Master Mix
0.5 µL	Ligation Enhancer

End-prep

32 **Instructions to End-prep reaction**

Note

This is a ‘one-pot ligation’ protocol for native barcoded ligation libraries.

Note

This step should be conducted in the PCR room

33 Add 2.5 µL of **Master Mix End-prep** to each tube containing 12.5 µL of pre-normalised DNA (~ 60ng for 2 kb) and mix well by pipetting.

34 Incubate in a thermocycler at 20 °C for 00:10:00 then 65 °C for 00:05:00

35 Place on ice for 00:00:30

Barcoding

25m

36

Instructions to Barcoding reaction

> Note
>
> Use a SINGLE native barcode (NBXX) per biological sample.

> Note
>
> This step should be conducted in the PCR room

37 Add 2.5 µL NBXX barcode to each tube containing 15 µL DNA end-prepped.

38 Add 18 µL of the barcoding mix to each tube and mix well by pipetting. The total volume will be 35.5 µL .

39 Incubate:

25m

20 °C 00:15:00

70 °C 00:10:00

Then place on ice.

> Note
>
> The 70 °C incubation is to inactivate the DNA ligase to prevent barcode cross-ligation when reactions are pooled in the next step

Pooling and clean-up

25m

40 **Instructions to pool all samples and perform a clean-up**

Pool all barcoded samples together into a clean 1.5 ml Eppendorf tube. 35 µL per sample

Note

If the number of samples processed is more than 20 samples this pooling needs to be done in a 2.0mL Eppendorf tube.

41 Add a proportion 1:1 of AMPURE beads in the barcoded DNA pooled.
If necessary measure the volume of the above reaction and add the same volume of AMPURE beads, to get a 1:1 solution.

42 Incubate for 00:05:00 in the HulaMixer.

43 Spin down the liquid and place on a magnet rack for 00:03:00 or until clear.

44 Remove all the solutions without touch in the pellet

45 Add 500 µL of [M] 80 % (v/v) ethanol to the tube still on the magnetic rack to wash.

46 Remove the tube from the magnet rack and remove and turn 180º and place the tube again in the magnetic rack.

47 Remove and discard [M] 80 % (v/v) ethanol without disturbing the pellet.

48 Repeat steps 45 and 47 and after continue from step 49.

49 Spin down and remove residual [M] 80 % (v/v) ethanol and air dry for 00:02:00 . 2m

50 Resuspend in 32 µL EB.

51 Incubate off the magnetic rack for 00:02:00 and after spin down. 2m

52 Replace the magnetic rack for 00:02:00

53 Wait until clear, then carefully remove the solution and transfer to a clean 1.5 mL Eppendorf tube labeled such as the name of your processing file.

QC

25m

54 Remove 2 µL and assess concentration by Qubit as described in the previous section. Keep the records in your processing file

Normalisation

25m

55 Dilute 100-200 fmol (~ 150ng for 2kb) pooled sample to 30 µL in EB.

Adapter ligation

25m

56 Set up the following adapter ligation reaction in a 1.5mL tube:

VOLUME	COMPONENT
30 µL	Cleaned-up barcoded amplicon pools (100-200 fmol)
10 µL	NEBNext Quick Ligation Reaction Buffer (5X)
5 µL	AMII Adapter mix II
5 µL	Quick T4 DNA Ligase

57 Incubate at RT for 00:30:00

Note

Remove the **SFB** from the freezer and let equilibrate to room temperature to be used in the next step

Clean-up

25m

58

Clean-up

After 00:30:00 incubation add 50 µL Ampure beads (1x the volume of the total reaction volume)

59 30 µL Incubate for 00:05:00

60 Spin down.

61 Place on a magnetic rack until clear.

62 Remove supernatant.

63 Add 200 µL **SFB**. Remove and turn the tube in the magnetic rack.

Note

CAUTION: **DO NOT USE** [M] 80 % (v/v) ETHANOL

64 Place on a magnetic rack until clear.

65 Remove supernatant.

66 Repeat **SFB** wash

67 Spin down and remove residual SFB .

Note

SFB is used for short fragments < 3kb.
LFB is used just for long fragments > 3Kb.

68 Add 14 µL EB and resuspend by flicking.

69 Incubate at RT for 00:02:00

70 Place on a magnetic rack.

71 Carefully transfer the solution to a clean 1.5 mL Eppendorf tube.

QC

25m

72 Remove 2 µL and assess concentration by Qubit fluorometer- recovery aim 50-100 fmol, 61.80 - 123.6 ng for 2Kb amplicons

Note

The library can be now stored at 4°C if required, but for best results, proceed immediately to sequencing.

Testing the flow cell

25m

73

Note

During this testing to save time you can thaw the following reagents at room temperature before placing on ice: Sequencing buffer (SQB); Loading beads (LB); Flush buffer (FB); and Flush tether (FLT). They will be used in the next step

Note

Choose a flow cell, record the name of the flow cell on your working sheet

74 Insert the flow cell in the MinION device or GridION.

75 Test the flow cell using the MinKnow software to observe the number of active pores, keep this record on your working sheet.

76 Select the flowcell ticking the box

77 Click “check flowcell”

78 Click “start test”.

Loading the flow cell

25m

79 For this step please follow the protocol described by Josh Quick below:

Citation

Josh Quick (2026). Priming and loading a MinION flowcell.

dx.doi.org/10.17504/protocols.io.7q5hmy6 LINK

MinKnow software

25m

80 Double–click the MinKNOW icon located on the desktop to open the MinKNOW software.

81 If your MinION was disconnected from the computer, plug it back in.

82 Choose the following flow cell type from the selector box: FLO-MIN106 : R9.4.1 flow cell

83 Then mark the flow cell as Selected.

84 Click the New Experiment button at the bottom left of the screen.

85 On the new experiment popup screen select the running parameters for your experiment from the individual tabs:

Experiment
On the New experiment popup screen, select the running parameters for your experiment from the individual tabs

Experiment: Name the run in the experiment field, leave the sample field blank.

Kit: Select LSK109. Do not select barcoding kits.

Run Options: Set the run length to 12 hours (you can stop the run once sufficient data has been collected as determined using RAMPART software).

Basecalling: Leave base calling turned but select 'high accuracy basecalling'.

Click 'Start run'.

Wash a flow cell

40m

86 **Washing the flow cell:**

Note

This step should be performed to reuse the flow cell after a previous run.

Note

The flow cell should be washed on the same day or on the following day of the run. Do not wait too much time to wash the Flow Cell.

For this step please follow the protocol described by Kirstyn Brunker bellow:

Citation

Kirstyn Brunker (2026). Washing a MinION flowcell.

dx.doi.org/10.17504/protocols.io.bddzi276 LINK

Citations

Quick J, Grubaugh ND, Pullan ST, Claro IM, Smith AD, Gangavarapu K, Oliveira G, Robles-Sikisaka R, Rogers TF, Beutler NA, Burton DR, Lewis-Ximenez LL, de Jesus JG, Giovanetti M, Hill SC, Black A, Bedford T, Carroll MW, Nunes M, Alcantara LC Jr, Sabino EC, Baylis SA, Faria NR, Loose M, Simpson JT, Pybus OG, Andersen KG, Loman NJ. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples.
https://doi.org/10.1038/nprot.2017.066

Step 79

Josh Quick. Priming and loading a MinION flowcell
dx.doi.org/10.17504/protocols.io.7q5hmy6

Step 86

Kirstyn Brunker. Washing a MinION flowcell
dx.doi.org/10.17504/protocols.io.bddzi276