'VirION': Long-read, low-input, viral metagenomic sequencing; Library Preparation and MinION (Oxford Nanopore Technologies) Sequencing (lib. prep. kit SQK-LSK108; flow cell: R9.4) v4

Joanna Warwick-Dugdale,Ben Temperton

Abstract

This protocol describes a generalizable, long-read, low-input metagenomic sequencing approach ('VirION') for the survey of viral communities. A significant obstacle in adopting long-read technology for viral metagenomics lies in obtaining the amount of DNA required; e.g. viral DNA extraction from 20 L of seawater yields far less than the micrograms of DNA recommended for efficient long-read sequencing. To overcome this limitation, we developed a Long-Read Linker-Amplified Shotgun Library approach for long-read viral metagenomics. The VirION method has been demonstrated to be as relatively quantitative as short-read methods, and analyses that combined VirION long-read data with Illumina, short-read data, captured many abundant and ubiquitous viral genomes that were missed by short-read assemblies. This approach was also shown to have overcome issues of microdiversity, and to have captured more genomic islands than short-read assemblies. Thus, VirION provides a high throughput and cost-effective alternative to fosmid and single-virus genomic approaches.

Citation: Joanna Warwick-Dugdale,Ben Temperton 'VirION': Long-read, low-input, viral metagenomic sequencing; Library Preparation and MinION (Oxford Nanopore Technologies) Sequencing (lib. prep. kit SQK-LSK108; flow cell: R9.4) v4. **protocols.io**

dx.doi.org/10.17504/protocols.io.p8fdrtn

Published: 12 Jun 2018

Guidelines

Method contents

A: DNA fragmentation
B: End-prep and clean-up
C: PCR adapter ligation and clean-up **(possible stop stage)**
D: Amplification and clean-up **(possible stop stage)**
E: 2nd End prep and clean-up
F: Adapter ligation
G: Adapter bead binding
H: Elution of library
I: Preparation of host computer and performing Flow cell QC
J: Priming the flow cell
K: Loading the library
L: Completing the experiment

Note: Process sheared, diluted commercial lambda DNA as a postivie control, through stages A-E, in order to verify success of amplification stages.

Equipment and consumables

- Vortex mixer
- Pipettes P1000, P200, P100, P20, P10 and P2
- Tips: 1000 µl, 200 µl, 10 µl
- Magnet for bead separation (a decent make, e.g. DynaMag™ Spin Magnet)
- Micro centrifuge *(model: Eppendorf 5424, if possible)
- Covaris g-TUBE (1 per sample, plus one for a positive control)
- 1.5 ml Eppendorf LoBind centrifuge tubes (Important: some plastics inhibit library prep.)
- 2 ml thin-walled PCR tubes
- Thermal cycler set for:
  - Program 1: 5 min at 20°C; 5 min at 65°C; 1 min 20°C; hold at 20°C
  - Program 2: 3 mins at 95°C; 15 cycles of: 15 secs at 95°C, 15 secs at 62°C, 5 min at 72°C, followed by one cycle of 5 min at 72°C; and hold at 4°C
- Qubit fluorimeter
- Qubit reaction tubes and Qubit dsDNA HS and BR Assay Kits
- Heat block set to 55°C
- Timer
- Filled Ice box
- Bioanalyzer/TapeStation
- Hula mixer (rotator: optional)
- Desktop/laptop:
  - Min. available memory: 50 GB
  - Sleeptimer and update turned off
  - For latest minimum computer specs: https://community.nanoporetech.com
- MinION Mk 1B

Reagents

- AMPure XP beads resuspended and at RT
- SQK-LSK108 sequencing kit (Oxford Nanopore)

Defrost following reagents on ice:

- ‘PCR Adapters’ (PCA),
- ‘Primers’ (PRM),
- ‘Running Buffer with Fuel Mix’ (RBF),
- 'Library Loading Beads' (LLB)‘
- Adapter Mix’ (AMX 1D) Note: leave in freezer until 10 min before needed

Defrost following reagents at RT:

- ‘Adapter Bead Binding’ (ABB)
- ‘Elusion Buffer’ (ELB)
- NEB Next Ultra II End-repair / dA-tailing Module (E7546S):
  - ‘Ultra II End-Prep Buffer’ - defrost at RT; check for precipitate; vortex well to dissolve if necessary
  - ‘Ultra II End-Prep Enzyme Mix’ - defrost on ice
- NEB Blunt / TA Ligase Master Mix (M0367S) – check for precipitate; take out of freezer 10 min before use and keep on ice
- NEB Next High-Fidelity 2X PCR Master Mix (Product number: M0541), take out of freezer ~15 min before use and put on ice
- Nuclease free water (NFW)

- Freshly prepared 80% EtOH (with NFW)
- Commercial lambda DNA sheared as detailed in stage ‘A’, and diluted to match sample concentration

Before start

Sample requirements

<1 µg of viral metagenomic DNA in 47 µl of elution buffer (EB) (e.g. 10 mM Tris-Cl, pH 8.5) or nuclease free water (NFW)

Important note: EDTA compromises flow cell pores, so do not use TE to re-suspend/elute viral DNA; If viruses were obtained by chemical flocculation and resuspension, further cleaning (post DNA extraction with Wizard DNA Clean-Up System) may be required (e.g. using DNeasy PowerClean Pro Kit (Cat No./ID: 12997-50).

Stopping stages

If pausing (e.g. overnight) either after PCR adapter ligation and clean-up (Stage ‘C’) or after amplification and clean-up (Stage ‘D’), some reagents will not be required immediately: check protocol to ensure reagents are not defrosted unnecessarily. Replace reagents taken from freezer to -20°C when they have been used and are no longer required (check later stages).

Protocol

A: DNA fragmentation

Step 1.
Ensure DNA is not stuck to tube by gently flicking; then spin down; transfer <1 µg DNA in 47 µl NFW/EB into the top compartment of a Covaris g-TUBE; screw on lid

A: DNA fragmentation

Step 2.
Shear DNA: Spin g-TUBE for 1 min at 6000 rpm (check all DNA has transferred into lower compartment of g-TUBE; if not, repeat 1 min spin); flip tube over (so base points upwards) and spin again for 1 min at 6000 rpm (check all DNA has transferred into lid of g-TUBE; if not, repeat 1 min spin);

NOTES

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Keep processing time <15 min;

*if a microcentrifuge other than model specified is used, spin speed may have to be optimised to obtain 8 Kbp fragments

A: DNA fragmentation

Step 3.

keep g-TUBE base upward, carefully unscrew tube and transfer DNA from lid to a fresh LoBind Eppendorf or (if proceeding directly to stage 'B') 0.2 ml PCR tube

Retain 1 µl of sheared DNA for later quantification via QuBit fluorimeter

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Assessment of fragment size Bioanalyzer/TapeStation not usually possible due to dilute nature of DNA

B: End-prep and clean-up

Step 4.

To 45 µl of fragmented and repaired DNA, add:

- 5 µl NFW
- 7 µl Ultra II End-Prep buffer
- 3 µl Ultra II End-Prep enzyme mix

(resulting in 60 µl total reaction volume)

B: End-prep and clean-up

Step 5.

Mix by gentle flicking, then spin down (transfer end-prep reaction to a 0.2 ml PCR tube, if needed)

B: End-prep and clean-up

Step 6.

Using thermocycler program 1, incubate for:

- 5 mins at 20 °C
- 5 min at 65 °C,
- 1 min 20 °C

Then transfer to fresh DNA LoBind tube

B: End-prep and clean-up

Step 7.

Resuspend AMPure beads (vortex); add 60 µl of beads to end-prep reaction; mix by gentle flicking

B: End-prep and clean-up

Step 8.

Incubate for 5 mins at RT, either on rotator, or gently flick every 1 min

TEMPERATURE

20 °C Additional info:

Room temp.

B: End-prep and clean-up

Step 9.

Spin down the sample and pellet on a magnet until the eluate is clear and colourless (2 min)

B: End-prep and clean-up

Step 10.

Leaving the tube on the magnet, carefully pipette off and discard the supernatant

B: End-prep and clean-up

Step 11.

Keep tube on magnet, and wash beads with 200 µl of freshly prepared 80% ethanol (without disturbing the pellet; leave 15 secs)

B: End-prep and clean-up

Step 12.

Remove the 80% ethanol using a pipette and discard

B: End-prep and clean-up

Step 13.

Repeat wash (steps 11 and 12)

B: End-prep and clean-up

Step 14.

Spin down briefly, replace on magnet, pipette off residual wash; briefly allow to dry (1-2 min)

NOTES

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It is important beads are allowed to dry to ensure all ethanol has been removed, however, over drying will result in some of the DNA not eluting efficiently leading to reduced recovery

B: End-prep and clean-up

Step 15.

Remove the tube from the magnetic rack and resuspend pellet in **31 µl** nuclease-free water by gentle flicking

B: End-prep and clean-up

Step 16.

Incubate for 2 minutes at 55 °C (heat block)

TEMPERATURE

55 °C Additional info: In heat block

B: End-prep and clean-up

Step 17.
Pellet beads on magnet until the eluate is clear and colourless (2 min)

B: End-prep and clean-up

Step 18.
Transfer eluate to fresh DNA LoBind tube;
Retain 1 µl of fragmented and end-prepped DNA for later quantification (QuBit fluorimeter)

NOTES

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Recovery aim: ≥ 20 ng (dependent on starting quantity).

C. PCR adapter ligation and clean-up

Step 19.
To 30 µl of end-prepped DNA, add:

- 20 µl of PCA
- 50 µl Blunt/TA Ligase Master Mix

(resulting in 100 µl total reaction volume)
Mix by gentle flicking, then spin down

NOTES

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Note: mix between each addition by gentle flicking, and spin down

C. PCR adapter ligation and clean-up

Step 20.
Incubate at RT for 10 minutes

TEMPERATURE

20 °C Additional info:
Room temp.

C. PCR adapter ligation and clean-up

Step 21.
Resuspend AMPure beads (vortex); add 40 µl of beads to PCR adapter reaction; mix by gentle flicking

NOTES

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This 0.4x beadwash removes small fragments of template DNA before PCR step

C. PCR adapter ligation and clean-up

Step 22.
Incubate for 5 mins at RT, either on rotator, or gently flick every 1 min

TEMPERATURE

20 °C Additional info:
Room temp.

C. PCR adapter ligation and clean-up

Step 23.
Spin down the sample and pellet on a magnet until the eluate is clear and colourless (3 min)

C. PCR adapter ligation and clean-up

Step 24.
Leaving the tube on the magnet, carefully pipette off and discard the supernatant

C. PCR adapter ligation and clean-up

Step 25.
Keep tube on magnet, and wash beads with 200 µl of freshly prepared 80% ethanol (without disturbing the pellet; leave 15 secs)

C. PCR adapter ligation and clean-up

Step 26.
Remove the 80% ethanol using a pipette and discard

C. PCR adapter ligation and clean-up

Step 27.
repeat wash (steps 25 and 26)

C. PCR adapter ligation and clean-up

Step 28.
Briefly spin down, replace on magnet, pipette of residual wash; briefly allow to dry (note importance of drying time, as above)

C. PCR adapter ligation and clean-up

Step 29.
Remove the tube from the magnetic rack and resuspend pellet in **26 µl** nuclease-free water by gentle flicking

C. PCR adapter ligation and clean-up

Step 30.
Incubate for 2 minutes at 55 °C (heat block)

TEMPERATURE

55 °C Additional info: Heat
block

C. PCR adapter ligation and clean-up

Step 31.

Pellet beads on magnet until the eluate is clear and colourless (3 min)

C. PCR adapter ligation and clean-up

Step 32.

Transfer eluate to fresh DNA LoBind tube

Conduct a High Sensitivity (HS) QuBit fluorimeter quantification on 1 µl of PCR adapted and ligated DNA (also quantify retained DNA from stages ‘A’ and ‘B’)

C. PCR adapter ligation and clean-up

Step 33.

Calculate volume required to add 20 ng of PCR-adapter ligated DNA into the PCR reaction below (e.g. 2.7 ng/µl x 8 = 21.6 ng; use 8 µl)

NOTES

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If not proceeding directly to stage ‘D’, PCR-adapter ligated DNA should be stored post clean-up at -20°C.

D. Amplification and clean-up

Step 34.

Set up the following PCR reactions (total volume 100 µl) in 0.2 ml thin-walled tubes, on ice:

- *40 µl NFW
- 2 µl PRM (primers)
- *8 µl PCR adapted and ligated DNA (from stage ‘C’)
- 50 µl NEB Next High Fidelity 2X master mix

:Mix by gentle flicking and spin down briefly

NOTES

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*Adjust volume of NFW and DNA as necessary for a 100µl reaction with ~20 ng of PCR-adapter ligated DNA, using the volumes of PRM and NEB Next master mix specified

Set up as many reactions as possible to increase yield of amplicons for sequencing; also set up the PCR-adapter ligated lambda as a positive control

D. Amplification and clean-up

Step 35.

Quickly transfer PCR reactions to the thermal cycler and run the following (Program 2):

- Initial denaturation: 3 mins at 95°C;
- 15 cycles of:
  - Denaturation: 15 secs at 95°C;

- Annealing: 15 at 62°C;
- Extension: 5 min at 72°C;

- Final Extension: 5 min at 72°C;
- Hold at 4°C

D. Amplification and clean-up

Step 36.

Spin down and transfer each reaction to a fresh DNA LoBind tube

D. Amplification and clean-up

Step 37.

Resuspend AMPure beads (vortex); add 40 µl of beads to each PCR reaction; mix by gentle flicking

NOTES

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This 0.4x beadwash removes small amplification products

D. Amplification and clean-up

Step 38.

Incubate for 5 mins at RT, either on rotator, or gently flick every 1 min

TEMPERATURE

20 °C Additional info:
Room temp.

D. Amplification and clean-up

Step 39.

Spin down the samples and pellet on a magnet until the eluates are clear and colourless (3 min)

D. Amplification and clean-up

Step 40.

Leaving the tubes on the magnet, carefully pipette off and discard the supernatant

D. Amplification and clean-up

Step 41.

Keep tubes on magnet, and wash beads with 200 µl of freshly prepared 80% ethanol (without disturbing the pellets; leave 15 secs)

D. Amplification and clean-up

Step 42.

Remove the 80% ethanol using a pipette and discard

D. Amplification and clean-up

Step 43.

Repeat wash (steps 41 & 42)

D. Amplification and clean-up

Step 44.

Briefly spin down, replace tubes on magnet, pipette of residual wash; briefly allow to dry (note importance of drying time, as above)

D. Amplification and clean-up

Step 45.

Remove the tubes from the magnetic rack and resuspend pellets in **26 µl** nuclease-free water by gentle flicking

D. Amplification and clean-up

Step 46.

Incubate for 2 minutes at 55°C (heat block)

TEMPERATURE

55 °C Additional info: Heat block

D. Amplification and clean-up

Step 47.

Pellet beads on magnet until the eluate is clear and colourless (3 min)

D. Amplification and clean-up

Step 48.

Transfer eluate to fresh DNA LoBind tube

Conduct Broad Range (BR) QuBit fluorimeter quantification on 1 µl of each PCR product

Conduct Bionalyzer/Tapestation assay on 1 µl of each PCR product

NOTES

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Check no high molecular weight product present in adapted and ligated PCR product via Tapestation/Bioanalyzer; very high MW product is associated with poor sequencing; if observed, repeat PCR with fewer cycles.

If not proceeding directly to stage ‘E’, PCR products should be stored post clean-up at -20°C.

E. 2nd End prep and clean-up

Step 49.

Concentrate (e.g. via SpeedVac) 3-3.5 µg of PCR product into 45 µl of NFW

E. 2nd End prep and clean-up

Step 50.

To 45 µl of PCR product in NFW add:

- 5 µl NFW
- 7 µl Ultra II End-Prep buffer
- 3 µl Ultra II End-Prep enzyme mix

(resulting in 60 µl total reaction volume)

E. 2nd End prep and clean-up

Step 51.

Mix by gentle flicking, then spin down; transfer end-prep reaction to a 0.2 ml PCR tube

E. 2nd End prep and clean-up

Step 52.

Using thermocycler program 1, incubate for:

- 5 mins at 20 °C
- 5 min at 65 °C,
- 1 min 20 °C

Then transfer to fresh DNA LoBind tube

E. 2nd End prep and clean-up

Step 53.

Resuspend AMPure beads (vortex); add 60 µl of beads to end-prep reaction; mix by gentle flicking

E. 2nd End prep and clean-up

Step 54.

Incubate for 5 mins at RT, either on rotator, or gently flick every 1 min

TEMPERATURE

20 °C Additional info:

Room temp.

E. 2nd End prep and clean-up

Step 55.

Spin down the sample and pellet on a magnet until the eluate is clear and colourless (2 min)

E. 2nd End prep and clean-up

Step 56.

Leaving the tube on the magnet, carefully pipette off and discard the supernatant

E. 2nd End prep and clean-up

Step 57.

Keep tube on magnet, and wash beads with 200 µl of freshly prepared 80% ethanol (without disturbing the pellet; leave 15 secs)

E. 2nd End prep and clean-up

Step 58.

Remove the 80% ethanol using a pipette and discard

E. 2nd End prep and clean-up

Step 59.

Repeat wash (steps 57 & 58)

E. 2nd End prep and clean-up

Step 60.

Spin down, replace on magnet, pipette of residual wash; briefly allow to dry (note importance of drying time, as above)

E. 2nd End prep and clean-up

Step 61.

Remove the tube from the magnetic rack and resuspend pellet in **31 µl** nuclease-free water by gentle flicking

E. 2nd End prep and clean-up

Step 62.

Incubate for 2 minutes at 55 °C (heat block)

TEMPERATURE

55 °C Additional info: Heat block

E. 2nd End prep and clean-up

Step 63.

Pellet beads on magnet until the eluate is clear and colourless (2 min)

E. 2nd End prep and clean-up

Step 64.

Transfer eluate to fresh DNA LoBind tube

Retain 1 µl of fragmented and repaired DNA for (later) quantification using a QuBit fluorimeter;

NOTES

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Recovery aim: >2500 ng.

F. Adapter ligation

Step 65.
To 30 µl of end-prepped amplified DNA, add:

- 20 µl AMX
- 50 µl NEB Blunt / TA Master Mix

(resulting in a final volume of 100 µl)
Mix by gentle flicking, then spin down

NOTES
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Mix between each addition by gentle flicking; spin down

F. Adapter ligation

Step 66.
Incubate at RT for 10 minutes

TEMPERATURE
20 °C Additional info:
Room temp.

G. Adapter bead binding

Step 67.
Resuspend AMPure beads (vortex); add 40 µl of beads to the adapter ligation reaction; mix by gentle flicking

G. Adapter bead binding

Step 68.
Incubate for 5 mins at RT, either on rotator, or gently flick every 1 min

TEMPERATURE
20 °C Additional info:
Room temp.

G. Adapter bead binding

Step 69.
Spin down the sample and pellet on a magnet until the eluate is clear and colourless (3 min)

G. Adapter bead binding

Step 70.
Leaving the tube on the magnet, carefully pipette off and discard the supernatant

G. Adapter bead binding

Step 71.

Add 140 µl of **ABB** to the beads; remove from magnet and resuspend by gentle flicking

G. Adapter bead binding

Step 72.

Pellet beads on magnet until the eluate is clear and colourless (5 min)

G. Adapter bead binding

Step 73.

Leaving the tube on the magnet, **very** carefully remove supernatant using a pipette and discard

G. Adapter bead binding

Step 74.

Repeat addition and removal of 140 µl of ABB (steps 71 -73)

H. Elution of library

Step 75.

Resuspend pelleted beads in **15 µl ELB** by gentle but tenacious flicking; incubate at RT for 10 minutes

TEMPERATURE

20 °C Additional info:

Room temp.

H. Elution of library

Step 76.

Pellet beads on magnet until the eluate is clear and colourless (5 min)

H. Elution of library

Step 77.

Remove and **retain 15 µl of eluate** into a clean 1.5 ml Eppendorf DNA LoBind tube

Place the tube of (adapted and tethered) library on ice until required for library loading

NOTES

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This elution will form the basis of the library loading mix

H. Elution of library

Step 78.

Conduct a second elution on the beads: add 15 µl ELB to the pellet; resuspend; incubate at RT for 10 min; pellet on magnet; remove and retain second eluate into clean 1.5. ml tube; place this on ice

NOTES

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If the first elution is somewhat low on DNA, this second one may form the basis of a second library loading mix. It may be worth loading the second library mix onto a flow cell at a point in the run where there does not appear to be enough DNA for the 'single pores' still available.

H. Elution of library

Step 79.

Quantify 1 µl of DNA library via QuBit fluorimeter (both elutions); also quantify DNA retained from previous stages at this time.

Recovery aim for RT eluate: >1000 ng (75ng/µl); ;

Remove flow cell from 4°C to RT 30 min before performing QC and loading library (check for bubbles on 'chip'; if bubbles noted, use alternative flow cell)

I. Preparation of host computer and performing Flow cell QC

Step 80.

On host computer ensure that:

- The internet is working;
- **>150 GB of memory is free on the C drive**;
- All sleep modes (including screensavers and log-offs) are disabled

I. Preparation of host computer and performing Flow cell QC

Step 81.

Connect MinION to host computer (light and fan operation indicates that the device is successfully plugged in); open MinKNOW software; Check for MinKNOW software updates

NOTES

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Check that the MinION configuration has been run prior to loading flow cell

I. Preparation of host computer and performing Flow cell QC

Step 82.

Insert flow cell into MinION (orientated with flow chip adjacent to hinge); close cover; check that MinKNOW is reporting a 'Flowcell connection' number and flowcell ID (if not, close MinKNOW, eject MinION, and repeat)

I. Preparation of host computer and performing Flow cell QC

Step 83.

Select running parameters:

- Label Experiment' and 'Sample ID' (as required)

- Select the appropriate operation (i.e. Platform QC)

I. Preparation of host computer and performing Flow cell QC

Step 84.
'Start' QC; to assist MinION gain correct temperature (approximately 34 °C), place MinION on insulating or conductive surface and modify airflow (as needed)

Allow script to run to completion (this will QC the flow cell; total time 10 min); note number of pore channels available (if <1000, consider using a different flow cell)

Proceed with preparation of priming buffer

J. Priming the flow cell

Step 85.
Mix RBF thoroughly by vortexing/pipetting; briefly spin down; prepare priming buffer by adding **576 µl** of RBF to **624 µl** NFW (total: 1200); mix by vortexing, spin down.

J. Priming the flow cell

Step 86.
Open the Minion lid and flow cell 'Priming Port' (turn clockwise)

J. Priming the flow cell

Step 87.
To ensure liquid-liquid contact between priming buffer flow cell buffer, and a continuous buffer flow from the Priming Port port across the sensor array:

- Place pipette tip of a Gilson 1000 µl pipette at flow cell Priming Port; hold vertically and slowly wind pipette (anticlockwise) to draw back a small volume of the (yellow) QC buffer from the flow cell (a few µls)
- Visually check that there is continuous buffer from the Priming Port across the sensor array; continue to wind pipette if necessary to remove any small bubbles, **but do not draw bubbles from post-sensor channel into the array**

J. Priming the flow cell

Step 88.
Avoiding the introduction of air bubbles, **load 800 µl of the priming mix** via the Priming Port: wind a vertical Gilson pipette (clockwise), use pipette plunger (with care) under 200-100 µl volume, and remove pipette with a few µls left in tip

J. Priming the flow cell

Step 89.
Proceed with preparing library for loading while flow cells 'primes' for 5 min

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Secondary priming of flow cell with **200 µl priming buffer** (see below) should take place immediately before loading the library

K. Loading the library

Step 90.

To a fresh low bind tube, add the following (in order):

- 35 µl RBF;
- 2.5 µl NFW;
- 25.5 µl LLB;
- 12 µl of adapted and tethered DNA library (i.e.: 'RT eluate')

(Total library loading mix: 75 µl)

Mix by gentle flicking (gently spin down if needed)

NOTES

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LLB will settle out very quickly; resupend by pipetting prior to transfter

K. Loading the library

Step 91.

Open the Spot-ON sample port cover with care

Very carefully and slowly, add 200 µl of **priming buffer** to the flow cell via the **Priming Port** (NOT the Spot-ON Sample Port), avoiding bubbles and maintaining liquid-liquid contact as before (including removal of a few µls beforehand if needed); proceed immediately to next step

K. Loading the library

Step 92.

Resuspend beads in the library loading mix via pipette, and immediately **load into the Spot-ON Sample Port in a dropwise fashion**; ensure each drop flows into the port before adding the next

K. Loading the library

Step 93.

Gently replace the Spot-ON Sample Port cover ensuring that the bung enters the port; close the Priming Port cover and replace the MinION lid

K. Loading the library

Step 94.

Wait for 1 hour prior to starting sequencing run

L. Starting the sequencing run

Step 95.
Complete experiment parameters:

- Choose correct library prep. kit (SQK-LSK108)
- Select correct flow cell (FLO-MIN106; R9.4)
- Switch **base calling to 'Off'**

(select other parameters as required; 48 hour run recommended)

L. Starting the sequencing run

Step 96.
Click on 'Begin Experiment' and:

- Note new number of pores reported in pre-sequencing QC ('Mux')
- Check pore occupancy figures (i.e. high ‘in-stand pore’ to ‘single pore’ ratio)
- Monitor messages in the Message panel in the MinKNOW GUI
- Allow the protocol to proceed in MinKNOW until it reports that the run is complete, or use ‘Stop’ in MinKNOW control panel to finish protocol

NOTES

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The read files are stored in :\data\reads (? ‘C\MinION_data\reads)

Base call reads after sequencing has been completed.