Laboratory Code of Practice (Containment Level 2/3)

Adapted from: Laboratory biosafety manual, 3rd Edition (World Health Organisation)

This Code of Practice should be read and understood by all persons working and entering Containment Level 2 (CL2) Labs. Any Group who creates their own, research/work specific Lab Code of Practice (COP) for CL2 work, should use this COP as a reference.

1. Eating, chewing, drinking, smoking, applying cosmetics/ lip salve, storing food or outdoor clothing, is not permitted in any Laboratories.

2. All workers in the laboratory must cover cuts and abrasions with a waterproof dressing.

4. Wash hands regularly and always before leaving the laboratory.

5. Laboratory coats, fully fastened with elasticated, close-fitting cuffs must be worn at all times whilst in the laboratory, and removed before leaving the laboratory.

6 Open toed shoes or sandals must not be worn in CL2 Laboratories.

7. Ensure that relevant training is received, completed to a competent level, and recorded, before use, for relevant scientific equipment.

8. The use of sharps should be minimised to as low as reasonably practicable. If sharps have to be used, then they must be placed directly into a sharps bin after use for safe disposal.

9. Latex gloves should not be used. Disposable gloves must not be reused. In the event of gloves becoming damaged or grossly contaminated the gloves must be discarded, hands washed, and new gloves put on.

10. Blood and animal tissues must only be handled at clearly identified, designated work stations. MSC's can be used as an easily managed containment area, or the use of a spill tray on lab bench can be considered.

11. All samples/containers/plates etc, which contain Biological Agents/Pathogens must be labelled with the name of the Biological Agent/Pathogen, and the initials of the researcher/ lab worker.

12. On completion of work, the work area and any relevant equipment used must be appropriately disinfected using a validated, biological agent-specific method.

13. Any infectious material transmissible by aerosol should be centrifuged in sealed safety buckets.

14. All biological or chemical waste materials must be made safe before disposal.

15. Following any spillage, surfaces must be disinfected with a suitable, validated disinfectant for the relevant contact time.

16. Accidents:

i) In the event of an accident resulting in a wound, immediately encourage it to bleed, wash thoroughly with soap and water, but DO NOT SCRUB. Cover with waterproof dressing.

ii) In the event of contamination of skin, conjunctivae or mucous membrane, immediately wash thoroughly. There are eye shower sprays and body showers available.

iii) Accidents must be reported to the Health & Safety Managers.

The above Code of Practice must be followed whilst in the laboratory.

Ghana-UK Partnership Complete Genome Sequencing of FMD
Protocol Part 1: Reverse transcription of RNA

This protocol performs reverse transcription of extracted Foot-and-Mouth-Disease Virus (FMDV) RNA into cDNA. It uses the Luna Script RT Supermix and the addition of a universal FMD primer to improve detection of FMD.

Protocol developed by Andrew Shaw, Edited by Bryan Wee and Erhan Yalcindag.

Starting Materials

- ❑ 7.5 µl of extracted RNA

Equipment

- ❑ P10 pipette (Micropipette with 0.5 – 10 µl range)
- ❑ PCR thermocycler (4°C - 95°C required)
- ❑ Microcentrifuge (for brief spin downs of 1.5ml Eppendorfs)
- ❑ (Optional) -20°C freezer for storage

Consumables

- ❑ P10 filter pipette tip (with 0.5 – 10 µl range)
- ❑ 0.1 ml 96-well PCR plates
- ❑ LunaScript RT Supermix (NEB, catalog no. M3010L)

Things to do before starting:

☐	• Clean well all PCR and sample preparation counter at regular intervals with a DNA degrading solution and/or Chemical Disinfection e.g. DNAZap™, 70% Ethanol.	
☐	• Prepare a 50 µM stock of FMD_UNI_RT, e.g. a 1:1 dilution of a 100 µM stock. (This will be provided for the workshop)	

☐	• Program the thermal cycler to run the following program (RT program):
	  ○ 24°C for 2 min
	  ○ 55°C for 10 min
	  ○ 95°C for 1 min
	  ○ Hold at 4°C
	• Do not start it yet.	

Important throughout the protocol:

-  Calculate necessary volume of final reagent mixture based off the number of samples plus an additional 2 (i.e. if 8 samples to be completed, multiply final volume of each individual PCR reagent by 10; if 10 samples, multiply by 12 etc. to ensure sufficient mastermix due to pipetting error.

Start of protocol

☐	• In a clean 0.1 ml PCR plate, prepare reverse transcription mix as follows: ○ 2 µl Lunascript RT supermix ○ 0.5 µl FMD_UNI_RT primer (50 µM) ○ 7.5 µl of your extracted RNA sample	
☐	• Mix by gentle flicking then pulse spin down for 2s in a microcentrifuge.	
☐	• Incubate the tubes in a thermocycler using the program (RT program) you have prepared. **Note:** You can store the resulting cDNA at -20°C at this stage if you like.	

Part 2: PCR amplification

This protocol performs two PCR reactions in parallel using primer pool A and primer pool B. This generates amplicons that eventually overlap and cover the whole FMD genome.

Protocol developed by Andrew Shaw, Edited by Bryan Wee and Erhan Yalcindag.

Materials

- ❑ cDNA from Part 1

Equipment

- ❑ P20 pipette (Micropipette with 2 – 20 µl range)
- ❑ P10 pipette (Micropipette with 0.5 – 10 µl range)
- ❑ Microcentrifuge (for brief spin downs of 1.5ml eppendorfs)
- ❑ PCR thermocycler (12°C - 98°C required)
- ❑ (Optional) -20°C or -4°C freezer for storage

Consumables

- ❑ Platinum™ SuperFi II PCR Master Mix – Green (Catalog No. 12369010)
- ❑ P20 filter pipette tips (with 2 – 20 µl range)
- ❑ P10 filter pipette tip (with 0.5 – 10 µl range)
- ❑ 0.1 ml 96-well PCR plates

Things to do before starting:

☐	• Prepare 100 µM stocks of each of the 28 primers. • Add equal volumes, e.g. 10 µl, of each 100 µM stock primer to its allocated pool, either pool A or pool B, according to the primer sheet, into a fresh tube. This is the 100 µM primer pool stock.	

	• Dilute each 100 µM primer pool stock 1:10 to generate pool A and pool B 10 µM working stocks.	

☐	• Program the thermal cycler to run the following program (PCR program):
	  ○ 98°C for 1 min
	  ○ 35 cycles of:
	  ▪ 98°C for 10 sec
	  ▪ 60°C for 15 sec
	  ▪ 72°C for 3 min
	  ○ 72°C for 5 min
	  ○ Hold at 12°C
	• Do not start it yet.	

Important throughout the protocol:

- Calculate necessary volume of final reagent mixture based off the number of samples plus an additional 2 (i.e. if 8 samples to be completed, multiply final volume of each individual PCR reagent by 10; if 10 samples, multiply by 12 etc. to ensure sufficient mastermix due to pipetting error.

Start of protocol:

☐ 1. For each sample, set up two PCR reactions as follows:

Component	Pool A reaction	Pool B reaction
Super Fi II 2x mastermix Green	5 µl	5 µl
Primer pool A	4 µl	-
Primer pool B	-	4 µl
cDNA from Part 1	1 µl	1 µl

Note: *Return excess cDNA to -20°C freezer*

☐ 2. Mix by gentle flicking then pulse spin down for 2s in a microcentrifuge.

☐ 3. Incubate the tubes in a thermocycler with the program (PCR program) you have prepared.

Note: *PCR samples can be left at this point overnight at 4 °C or stored longer term at -20°C.*

Part 3: Library preparation for Rapid Sequencing DNA V14 Barcoding kit (SQK-RBK114.24)

This protocol prepares Nanopore sequencing libraries for the MinION flow cell and is an edited version of the SQK-RBK114.24 protocol.

Protocol adapted from ONT. Adapted for FMD by Andrew Shaw. Edited by Bryan Wee and Erhan Yalcindag.

Material

- ❑ Amplified DNA from Part 2

Equipment

- ❑ P1000 pipette (Micropipette with 100 – 1000 µl range)
- ❑ P200 pipette (Micropipette with 20– 200 µl range)
- ❑ P20 pipette (Micropipette with 2 – 20 µl range)
- ❑ P10 pipette (Micropipette with 0.5 – 10 µl range)
- ❑ PCR thermocycler (21°C - 80°C required)
- ❑ DNA fluorometer (Thermofisher Qubit or alternatively Promega Quantus)
- ❑ Timer
- ❑ Magnetic rack
- ❑ Vortex mixer

Consumables

- ❑ P1000 filter pipette tips (with 100 – 1000 µl range)
- ❑ P200 filter pipette tips (with 20– 200 µl range)
- ❑ P20 filter pipette tips (with 2 – 20 µl range)
- ❑ P10 filter pipette tip (with 0.5 – 10 µl range)
- ❑ 0.1 ml 96-well PCR plates
- ❑ 1.5 ml Eppendorf DNA LoBind tubes
- ❑ Nuclease free water (NFW)
- ❑ 2.4ml ethanol
- ❑ 600 µl Nuclease Free Water
- ❑ Qubit dsDNA HS Assay Kit (Invitrogen, Q32851)
- ❑ Nanopore Rapid sequencing V14 - Amplicon sequencing (SQK-RBK114.24)
    - Rapid Adapter (RA)
    - Adapter Buffer (ADB)
    - AMPure XP Beads (AXP)
    - Elution Buffer (EB)
    - Sequencing Buffer (SB)
    - Library Beads (LIB)
    - Flow Cell Flush (FCF)
    - Flow Cell Tether (FCT)
    - Rapid Barcodes x 24 (RB01-24)
- ❑ Nanopore 10.4.1 MinION Flow Cell
- ❑ Frozen ice block
- ❑ DNAzap

Important throughout the protocol:

- From this point onwards perform the actions in a lab/zone dedicated for post-PCR work. Decontaminate this area along with pipettes and equipment frequently with DNAzap or equivalent cleaning reagents.

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Be gentle when pipetting, too much fast pipetting/ extended vortexing can shear the DNA which will result in poorer sequencing outcomes.
-  Do not let the beads dry after removal of supernatant. This can be avoided by keeping Eppendorf tubes closed if beads are not submerged. Note: When bead pellet is moist, it appears shiny. As they start to dry, the shine reduces, and cracks start to form.
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When working with beads ensure they are thoroughly mixed before using. This can be achieved by vortexing for at least 10 seconds
-  Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores. Practice pipetting on a used flow cell if possible.

Things to do before starting:

☐	1. Combine PCR products from reaction A and reaction B and mix thoroughly.	
☐	2. Assign each sample to a Barcode (1-24) and note this down.	
☐	3. Program the thermal cycler to incubate at 30°C for 2 min then 80°C for another 2 min. Do not start it yet.	
☐	4. Thaw kit components at room temperature, spin down briefly using a microfuge and mix by pipetting as indicated by the table below:	

Reagent	Thaw	Mix
Rapid Barcodes (RB01-24) or Rapid Barcode Plate (RB01-96)	Not frozen	Mix well by pipetting
Rapid Adapter (RA)	Not frozen	Vortex immediately before use
AMPure XP Beads (AXP)	Yes	Vortex immediately before use
Elution Buffer (EB)	Yes	Mix well by pipetting
Adapter Buffer (ADB)	Yes	Mix by vortexing

5.

Start of protocol:

☐	6. Note down the number of each 0.1 ml PCR plate well for each sample from the previous step. Allocate and note down the well corresponding to each barcode number.	
☐ ☐	7. In each 0.1 ml thin-walled PCR plate well: ○ 9 µl nuclease free water	

☐	○ 1 µl of mixed PCR products ○ 1 µl of your chosen Rapid Barcode (RB01-24). (1 barcode per sample) **Note**: *Spin barcodes down for 2-3 seconds before use*	
☐	8. Mix by gently by stirring or pipetting until thoroughly mixed. If there are any bubbles present, spin down briefly (2-3 seconds) to ensure all liquid is at the bottom. **Note:** *Barcodes will be a thicker liquid, visually check they have been mixed in.*	
☐	9. Incubate the tubes in the thermal cycler (PCR machine) at 30°C for 2 min then 80°C for another 2 min.	
☐	10. Briefly place the tubes on ice to cool. **Note:** *You can store the resulting cDNA overnight at 4°C at this stage if necessary.*	
☐	11. Pool all your samples into a 1.5ml clean Eppendorf DNA LoBind tube. Note down the total volume after pooling. It should be 11µl multiplied by the number of samples.	
☐	**Only 1 person is required to carry out the following steps.**	
☐	12. Resuspend the AMPure XP (AXP) beads by vortexing for 10 seconds or longer.	
☐	Use a 1:1 ratio of sample to AXP beads and mix into the sample by flicking the tube. For example, for 12 samples, add 132 µl of AXP to the 132 µl pooled barcoded DNA (12 x 11 = 132). **Note:** *If sticky clumps of bead-bound DNA form, be careful not to take any beads either in the pipette tip or on the outside of the pipette tip.*	
☐	13. Manually invert the sample back and forth for 10 minutes at room temperature for 10 min.	
	To do while waiting • Take out Elution Buffer (EB) to thaw (on ice block) • Prepare fresh 80% ethanol: ○ 2.4 mL 100% ethanol ○ 600 µl Nuclease Free Water (NFW)	
	14. Spin down the sample for 2-3 seconds in a microcentrifuge.	
☐	15. Place the sample on a magnetic stand for 2 min until the solution becomes clear and the beads form a pellet on one side of the tube.	
☐	16. While leaving the tube on the magnet, carefully remove and discard **supernatant** without disturbing the beads.	
☐	17. Wash 1: While still on the magnetic stand, carefully add 1 ml of freshly prepared 80% ethanol without flushing directly onto the pellet.	
☐	18. Leaving the tube on the magnet, remove the ethanol using a pipette and discard, without disturbing the beads	

☐ 19. Wash 2: While still on the magnetic stand, carefully add 1 ml of freshly prepared 80% ethanol without flushing directly onto the pellet.

☐ 20. Leaving the tube on the magnet, slowly remove the ethanol using a pipette and discard, without disturbing the beads

21. Briefly spin down for 2-3 seconds and place tube back on the magnet. Pipette off any residual ethanol with a P10/P20 pipette.

☐ 22. Allow the sample to air dry with lids open for 30 seconds watching it until the pellet is no longer shiny. Do not dry to the point of cracks appearing on the pellet.

☐ *23.* Remove the tube from the magnetic rack and add 15ul of Elution Buffer per 24 barcodes used. (Elution buffer (EB), included in the Nanopore Rapid Barcoding kit).
Resuspend the beads by slowly pipetting or stirring with the pipette tip.
Note: *Be as gentle as possible while ensuring that pellet is resuspended. Spin Elution buffer for 2-3 seconds.*

	24 barcodes	48 barcodes	72 barcodes	96 barcodes
Volume of Elution Buffer	15 µl	30 µl	45 µl	60 µl

☐ 24. Incubate the samples at room temperature for 10 minutes to elute the DNA.

☐ 25. Return the tube to the magnetic stand for at least 1 minute until the solution becomes clear and the beads form a pellet.

☐ *26.* Transfer **14** µl of the eluate into a clean 1.5ml Eppendorf DNA LoBind tube.
Note: *Be careful not to take any beads either in the pipette tip or on the outside of the pipette tip.*

☐ 27. Take 1µl of the elute from the 1.5ml Eppendorf tube on the for quantification on a fluorometer (Qubit or Quantus).

Refer to **Part 5** of the protocol.

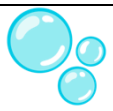
Note: *Samples can be left at this point overnight at 4 °C.*

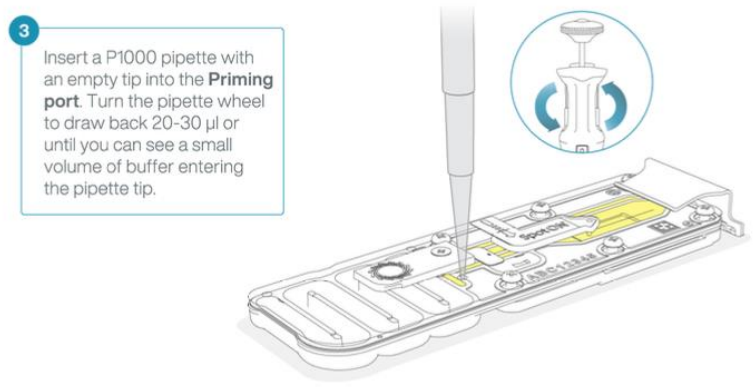
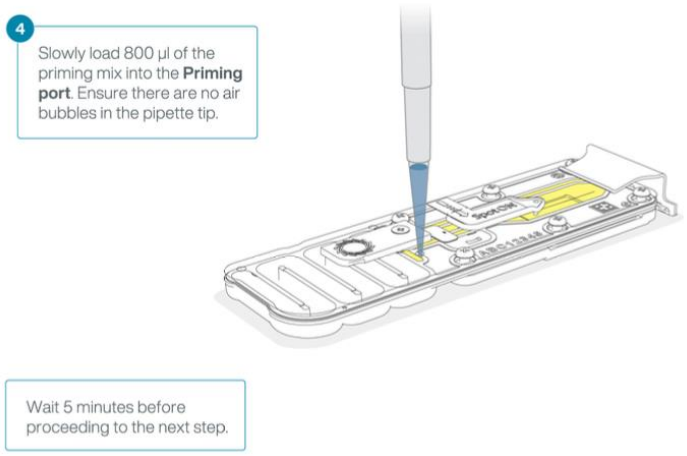
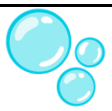
Adding the Rapid Adaptors

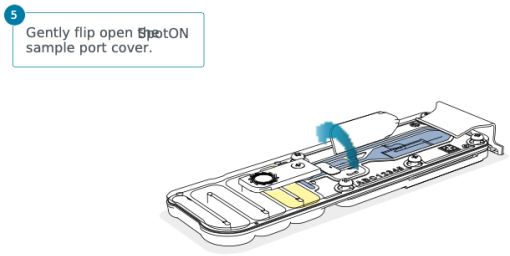
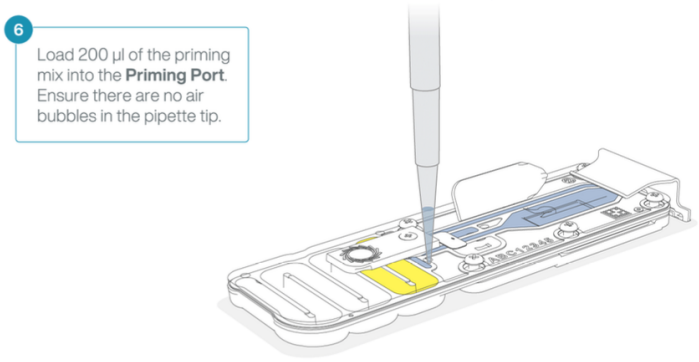
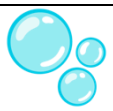
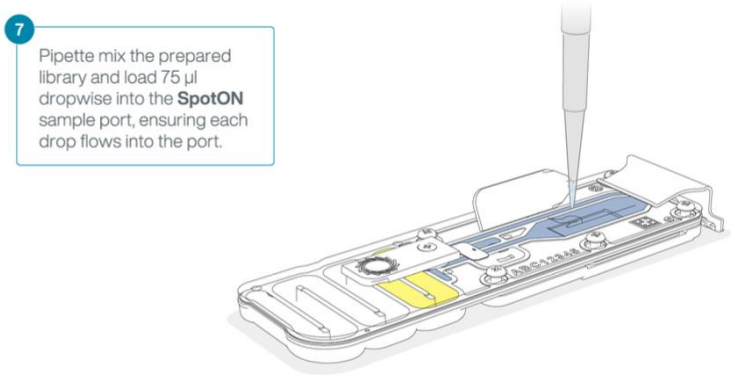
☐ 28. Transfer 11µl of DNA into a clean 1.5 ml Eppendorf DNA LoBind tube and dilute, if necessary, as follows:

Reagent	DNA library < 73 ng/µl	DNA library >73 ng/µl
DNA	11 µl	x µl containing 800ng (x =800/concentration)
EB	-	Adjust to 11 µl (=11-x)

☐ ☐ 29. In a new 1.5 ml Eppendorf DNA LoBind tube, mix the following:
- 1.5µl Rapid Adapter (RA)

☐	○ 3.5µl Adapter Buffer (ADB) **Note:** *Spin down the Rapid Adapter and Adapter Buffer*	
☐	30. Add 1µl of this RA + ADB mixture to the DNA	
☐	31. Mix gently by flicking and spin down briefly (2-3 seconds)	
☐	32. Incubate this for 5 mins at room temperature then place on ice block until ready to load the flowcell.	
	Priming and loading the flowcell A video is available demonstrating the steps described below: **https://community.nanoporetech.com/nanopore_learning/lessons/priming-and-loading-your-flow-cell**	
☐	33. Remove the following Nanopore Rapid Kit (RBK-114.24) items from the -20 °C freezer, spin down and store on the ice block. ○ SB (Sequencing Buffer) ○ LIB (Library Beads) ○ FCT (Flow Cell Tether) ○ FCF (Flow Cell Flush) ○ Bovine Serum Albumin (BSA) at 50mg/ml	
☐	34. Prepare the flow cell Priming Mix in a fresh 1.5ml Eppendorf DNA LoBind. Mix by inverting the tube. ○ 1,170µl FCF ○ 5 µl BSA (Bovine Serum Albumin) ○ 30 µl FCT	
☐	35. Remove the flow cell you want to use and slide it under the metal clip in the Mk1D or Mk1C MinION. Press down firmly to ensure correct contact with the thermal and electrical connections. ○ Mk1D: Plug in the MinION to a laptop with Minknow software ○ Mk1C: Turn on the MinION Mk1C.	
☐	36. Complete a flow cell check to assess the number of pores available on the flow cell.	
☐	37. Rotate the flow cell priming port cover clockwise 90 degrees to open the priming port.	
☐ ☐ ☐ ☐	38. After opening the priming port there will be a small air bubble under the cover that needs to be removed. ○ Set a P1000 pipette to 200 µl ○ Insert the tip into the priming port ○ Turn the adjustment wheel slowly, pausing every few µls, until the pipette shows 220 µl - 230 µl to draw a total of 20-30 µl out of the priming port, or until you can see a small volume of liquid entering the pipette tip. **Note 1:** *There may be a small delay before the liquid comes out of the port into the pipette tip. Do not draw out more than 30 µl.* **Note 2:** *Check that there is a continuous flow of buffer from the priming port to the nanopore sensor array, and that no bubbles are*	

	present. 	
☐	39. Load slowly 800µl of the Priming Mix from Step 33 into the priming port, without introducing any bubbles. Leave for 5 min and while waiting, proceed to the next step. 	
☐	40. Mix the LIB (Library Beads) thoroughly by pipetting.	
☐ ☐ ☐ ☐	41. In a new 1.5 ml LoBind tube, mix the following to prepare your library: ○ 37.5 µl SB (Sequencing Buffer) ○ 25.5 µl LIB (Library Beads), mixed immediately before use ○ 12 µl of DNA library (your sample)	
☐	42. Gently open the SpotON sample port cover.	

☐	43. Load slowly 200 µl of the Priming Mix into the priming port (**not the SpotON sample port**). Again, avoid introducing any air bubbles.  	
☐	44. Mix the prepared library by gently pipetting just prior to loading drop by drop 75 µl of the prepared library from Step 40 onto the SpotON sample port. Let each drop be "sucked" into the port before adding the next drop. 	
☐	45. Replace the SpotON sample port cover and gently press down to ensure the bung is in the port.	
☐	46. Rotate the Priming port cover back to close the Priming port.	
☐	47. Immediately install the light shield and close the MinION device lid.	
☐	48. Start the run on the Minknow software interface.	

Part 4: Flow Cell Wash (Kit EXP-WSH004 or EXP-WSH004-XL)

Protocol edited by Erhan Yalcindag, Andrew Shaw and Bryan Wee.

Materials

- ❑ Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL)

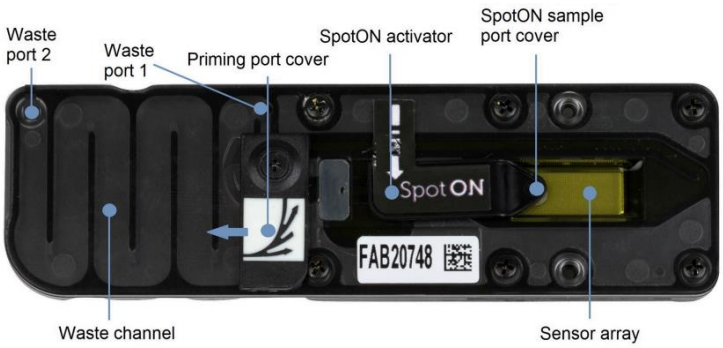
Equipment

- ❑ P1000 filter pipette tips (with 100 – 1000 µl range)
- ❑ P200 filter pipette tips (with 20– 200 µl range)
- ❑ Frozen ice block

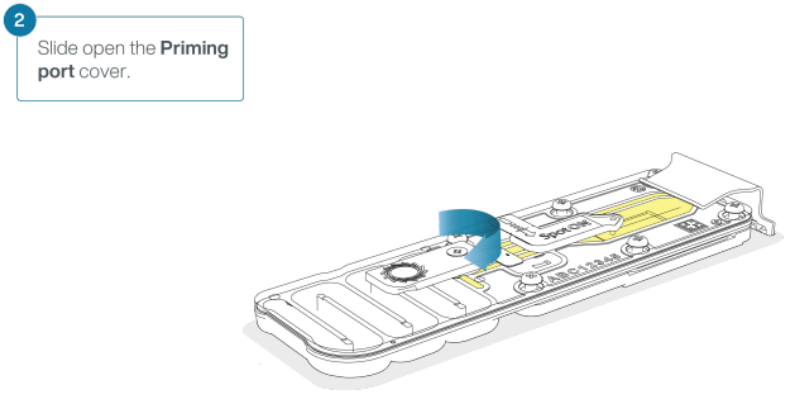
Preparation to run the washing procedure

- This protocol assumes that the flow cell has already had a DNA/RNA library run on it
- The aim is to remove most of this initial library from the flow cell
- The Wash Kit contains all solutions required for removal of the initial library
- After the flow cell has been washed, a new library can be loaded or the flow cell can be stored at 4°C

☐	1. Place the tube of Wash Mix (WMX) on ice. Do not vortex the tube.	
☐	2. Thaw one tube of Wash Diluent (DIL) at room temperature.	
☐	3. Mix the contents of Wash Diluent (DIL) thoroughly by vortexing, then spin down briefly and place on ice.	
☐	4. In a fresh 1.5 ml Eppendorf DNA LoBind Eppendorf 1.5 ml tube, prepare the following Flow Cell Wash Mix: Reagent | **Volume per Flowcell** Wash Mix (WMX) | 398 µl Wash Diluent (DIL) | 2 µl Total | 400- µl	
☐	5. Mix well by pipetting, and place on ice. Do not vortex the tube.	
☐	6. Stop or pause the sequencing experiment in MinKNOW, and leave the flow cell in the device.	
☐	7. Remove the waste buffer, as follows: a. Close the priming port and SpotON sample port cover, as indicated in the figure below. b. Insert a P1000 pipette into waste port 1 and remove the waste buffer. **Note:** *As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.*	

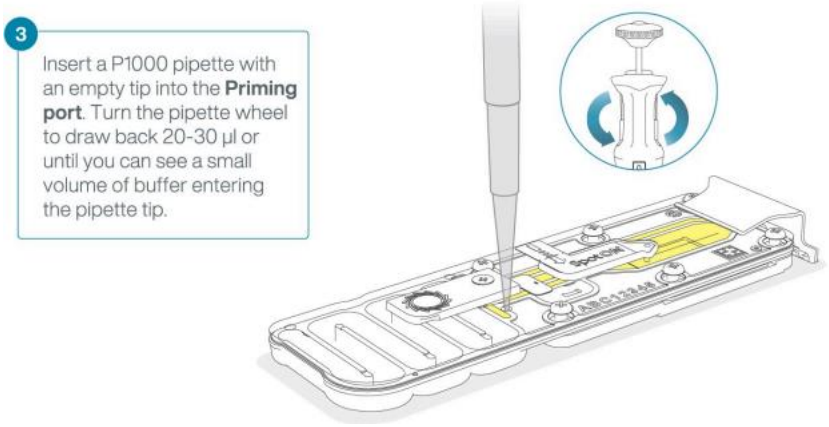


☐ 8. Slide the flow cell priming port cover clockwise to open.

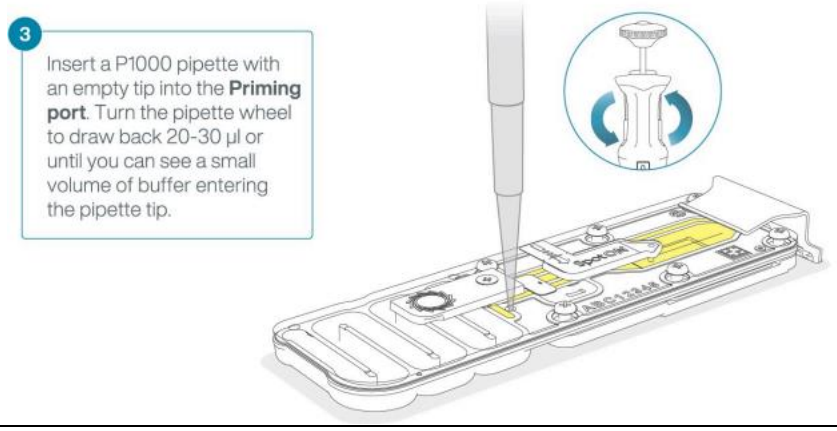
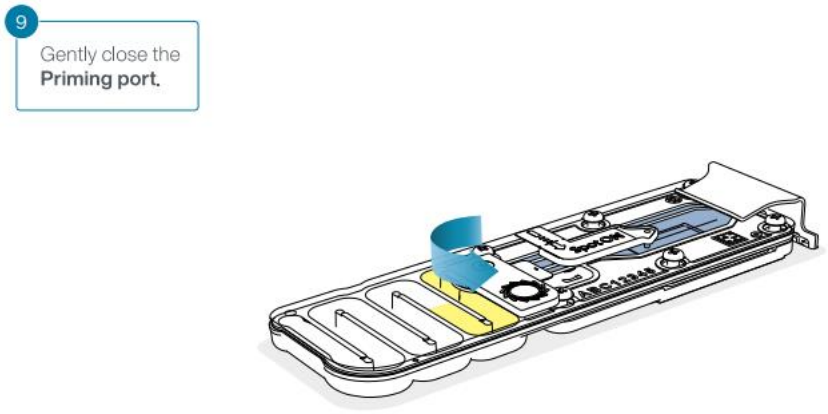


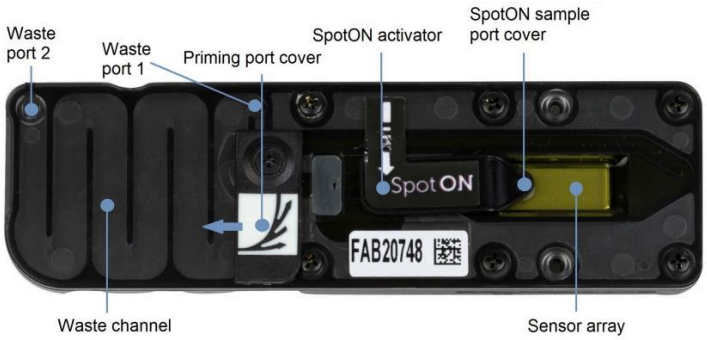
☐ 9. After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:

a. Set a P1000 pipette to 200 µl.
b. Insert the tip into the ow cell priming port.
c. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer/liquid entering the pipette tip.
d. Visually check that there is continuous buffer from the flow cell priming port across the sensor array.



Note: *Take care when drawing back buffer from the flow cell. Do not remove more than 20-30µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.*

☐	10. Slowly load 200µl of the prepared ow cell wash mix into the priming port, as follows: a. Using a P1000 pipette, take 200 µl of the ow cell wash mix b. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip c. Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger very slowly, leaving a small volume of buffer in the pipette tip d. Set a timer for a 5 minute incubation. 	
☐	11. Once the 5 minute incubation is complete, carefully load the remaining 200µl of the prepared flow cell wash mix into the priming port, as follows: a. Using a P1000 pipette, take the remaining 200 µl of the flow cell wash mix b. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip c. Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger **very slowly**, leaving a small volume of buffer in the pipette tip.	
☐	12. Close the priming port and wait for 1 hour. 	
☐	13. Remove the waste buffer, as follows a. Ensure the priming port and SpotON sample port covers are closed, as indicated in the figure below.	

	b. Insert a P1000 pipette into waste port 1 and remove the waste buffer. **Note:** *As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.*  	
☐	14. End of Step a. Follow one of the two options described in the next steps of the protocol. b. Run a second library on the ow cell straight away	
☐	15. Store the flow cell at 8°C for later use	

Part 5: DNA Quantification using the Qubit HS dsSNA kit

This protocol enables the quantification of DNA during the library preparation step to ensure correct dilution for rapid adaptors.

Protocol edited by Erhan Yalcindag, Andrew Shaw and Bryan Wee.

Materials

- ❑ Qubit dsDNA HS quantification Assay kits (Invitrogen, Q32851)

Equipment

- ❑ Qubit Fluorometer (Invitrogen, Q33238)
- ❑ 0.5 ml thin wall, clear, PCR tubes (Invitrogen, Q32856) or Axygen PCR-05-C tubes (part no. 10011-830).
- ❑ 1.5 ml Eppendorf DNA LoBind tubes
- ❑ P1000 pipette (Micropipette with 100 – 1000 µl range)
- ❑ P200 pipette (Micropipette with 20– 200 µl range)
- ❑ P20 pipette (Micropipette with 2 – 20 µl range)

- ❑ P10 pipette (Micropipette with 0.5 – 10 µl range)
- ❑ P1000 filter pipette tips (with 100 – 1000 µl range)
- ❑ P200 filter pipette tips (with 20– 200 µl range)
- ❑ P20 filter pipette tips (with 2 – 20 µl range)
- ❑ P10 filter pipette tip (with 0.5 – 10 µl range)

Start of protocol:

☐	1. Set up the required number of 0.5 ml tubes for standards and samples. The Qubit dsDNA HS Assay requires 2 standards. **Note:** *Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. no. Q32856) or Axygen PCR-05-C tubes (part no. 10011-830).*	
☐	2. 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.	
☐	3. Prepare the Qubit working solution by diluting the Qubit® dsDNA HS Reagent 1:200 in Qubit dsDNA HS Buffer. Use a clean plastic tube each time you prepare Qubit working solution.	
☐	4. The final volume in each tube must be 200 µl. Each standard tube requires 190 µl of Qubit working solution, and each sample tube requires anywhere from 180–199 µl. Be sure to prepare sufficient Qubit working solution to accommodate all standards and samples. For instance, for 4 samples, prepare enough working solution for the samples and 2 standards: ~200 µl per tube in 5 tubes yields 1 ml of working solution (5 µl of Qubit reagent plus 995 µl of Qubit buffer).	
☐	5. Add 190 µl of Qubit working solution to each of the tubes used for standards.	
☐	6. Add 10 µl of each Qubit standard to the appropriate tube, then mix by vortexing 2–3 seconds. Do not to create bubbles. Careful pipetting is critical to ensure that exactly 10 µl of each Qubit standard is added to 190 µL of Qubit working solution.	
☐	7. Add 1 µl of sample to each corresponding tube then add 199 µl of Qubit working solution to each assay tube so the final volume in each tube after adding sample is 200 µl.	
☐	8. Add each sample to the assay tubes containing the correct volume of Qubit working solution, then mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µl.	
☐	9. Allow all tubes to incubate at room temperature for 2 minutes.	
☐	10. On the main menu of the Qubit® 3.0 Fluorometer, press DNA, then select dsDNA High Sensitivity as the assay type. The “read standards” screen is displayed, press “read standards” to proceed.	
☐	11. Insert the tube containing Standard #1 into the sample chamber, close the lid, then press Read standard. When the reading is complete (~3 seconds), remove Standard #1.	
☐	12. Repeat above step for Standard #2. The instrument displays the results on the Read standard screen.	

☐	13. Press Run samples. On the assay screen, select the sample volume and units: a. Press the + or – buttons on the wheel to select a sample volume of 1 µl. b. From the dropdown menu, select the units for the output sample concentration.	
☐	14. Insert a sample tube into the sample chamber, close the lid, then press Read tube. When the reading is complete, remove the sample tube.	
☐	15. Repeat previous step until all samples have been read.	