Flow Cell Number: ..... DNA Samples: .....

Before start checklist

Materials

- [ ] 1 µg (or 100-200 fmol) high molecular weight genomic DNA
- [ ] OR 100+ ng high molecular weight genomic DNA if performing DNA fragmentation
- [ ] Ligation Sequencing Kit XL V14 (SQK-LSK114-XL)

Consumables

- [ ] NEBNext® Companion Module for Oxford Nanopore Technologies® Ligation Sequencing (NEB, E7180S or E7180L). Alternatively, you can use the NEBNext® products below:
- [ ] NEBNext FFPE Repair Mix (NEB, M6630)
- [ ] NEBNext Ultra II End repair/dA-tailing Module (NEB, E7546)
- [ ] NEBNext Quick Ligation Module (NEB, E6056)
- [ ] Agencourt AMPure XP Beads (Beckman Coulter™, A63881)
- [ ] Nuclease-free water (e.g. ThermoFisher, AM9937)
- [ ] Freshly prepared 80% ethanol in nuclease-free water
- [ ] Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, AM2616)
- [ ] Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, red/clear (Bio-Rad™, cat # HSP9611)
- [ ] Thermo Scientific™ Abgene™ 96 Well 1.2 ml Polypropylene Deepwell Storage Plate (Thermo Scientific, cat # AB1127)
- [ ] 1000 µl Disposable Conductive Tips - Liquid Handling Flexible Channel Arm - Filtered, Pure, ANSI/SLAS-format box (same as SBS) (Tecan , cat# 30057817)
- [ ] 200 ul Disposable Conductive Tips - Liquid Handling Flexible Channel Arm - Filtered, Pure, ANSI/SLAS-format box (same as SBS) (Tecan , cat# 30057815)
- [ ] 50 ul Disposable Conductive Tips - Liquid Handling Flexible Channel Arm - Filtered, Pure, ANSI/SLAS-format box (same as SBS) (Tecan , cat# 30057813)
- [ ] Small SBS Box to place conductive tips & refill, compatible with 10uL, 50uL, 200uL tips (Tecan , cat# 30058506)
- [ ] Big SBS Box to place conductive tips & refill, compatible with 1000uL tips (Tecan , cat# 30058507)
- [ ] 150 µl Disposable Tips - MultiChannel Arm™

Equipment

- [ ] Ice bucket with ice
- [ ] Vortex mixer
- [ ] Microplate centrifuge, e.g. Fisherbrand™ Mini Plate Spinner Centrifuge (Fisher Scientific, 11766427)
- [ ] Tecan DreamPrep NGS workstation with full configuration

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INSTRUCTIONS	NOTES/OBSERVATIONS
Library preparation	
Prepare the NEBNext FFPE DNA Repair Mix and NEBNext Ultra II End Repair / dA-tailing Module reagents in accordance with manufacturer's instructions, and place on ice. ☐ Thaw all reagents on ice. ☐ Flick and/or invert the reagent tubes to ensure they are well mixed. Note: Do not vortex the FFPE DNA Repair Mix or Ultra II End Prep Enzyme Mix. ☐ Always spin down tubes before opening for the first time each day. ☐ The Ultra II End Prep Buffer and FFPE DNA Repair Buffer may have a little precipitate. Allow the mixture to come to RT and pipette the buffer up and down several times to break up the precipitate, followed by vortexing the tube for 30 seconds to solubilise any precipitate. Note: It is important the buffers are mixed well by vortexing. ☐ The FFPE DNA Repair Buffer may have a yellow tinge and is fine to use if yellow. ☐ Switch on the Tecan DreamPrep NGS robot and open the Fluent Control software on the computer. Follow the recommended specifications to initiate the DreamPrep NGS.	
IMPORTANT ☐ Perform the 'Daily System Care' method to prepare the instrument before the first run of the day.	
☐ Users will have access to the 'Main screen' of TouchTools™, which allows interaction with the DreamPrep NGS system. Select 'Method Starter'. ☐ In the 'Method Starter' folder, select the Ligation sequencing program and click 'Ok'. ☐ Click the 'start button' in the middle of the screen to start the run. During the run set-up on the Tecan DreamPrep NGS, the workstation will perform automated checks and set-up for the ODTC. ☐ When you see the 'Welcome to the ONT Ligation Sequencing protocol by Tecan' page, click 'Continue'. ☐ Set the 'User defined variables' and click on 'Next page' to proceed. Set the 'User inputs' and click on 'Next page' to proceed. ☐ Please note that if removing the sample plate for off-deck storage, user interaction is required approximately 10 minutes after starting the run. ☐ We recommend RT for the majority of users. However, 37°C can be beneficial for recovery of longer DNA strands. ☐ The MinION option is valid for both the MinION and GridION device. ☐ Select if you would like to maintain the standard LSK volumes and timers as recommended by ONT and click on 'Next page' to proceed.	
IMPORTANT ☐ We highly recommend using default settings developed by Oxford Nanopore Technologies.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Review the run variables and click 'Confirm': Follow the on-screen directions to load the 150 µl filtered tips for the Multichannel Arm 384/96 onto the worktable: ☐ The required loading position for each box will flash to indicate where to place the labware. ☐ Please load full tip boxes only, partially full boxes of filtered tips for the Multichannel Arm 384/96 are currently not supported. ☐ Click 'Approve' after each addition of labware to proceed to the next box. ☐ After loading all of the required labware, close the front safety shield and click 'Next Page' to proceed. Follow the on-screen directions to load the 50 µl filtered tips for the Multichannel Arm 384/96 onto the worktable: ☐ The required loading position for each box will flash to indicate where to place the labware. ☐ Please load full tip boxes only, partially full boxes of filtered tips for the Multichannel Arm 384/96 are currently not supported. ☐ Click 'Approve' after each addition of labware to proceed to the next box. ☐ After loading all of the required labware, close the front safety shield and click 'Next Page' to proceed. Follow the on-screen directions to load the 1000 µl Flexible Channel Arm filtered tips onto the worktable: ☐ The required loading position for each box will flash to indicate where to place the labware. ☐ Partially full boxes are supported for the Flexible Channel Arm filtered tips. However, please ensure the tip box contains the minimum required number of tips as described in the equipment and consumables section of this protocol. ☐ Click 'Approve' after each addition of labware to proceed to the next box. Follow the on-screen directions to load the 200 µl Flexible Channel Arm filtered tips onto the worktable: ☐ The required loading position for each box will flash to indicate where to place the labware. ☐ Partially full boxes are supported for the Flexible Channel Arm filtered tips. However, please ensure the tip box contains the minimum required number of tips as described in the equipment and consumables section of this protocol. ☐ Click 'Approve' after each addition of labware to proceed to the next box. Follow the on-screen directions to load the 50 µl Flexible Channel Arm filtered tips onto the worktable: ☐ The required loading position for each box will flash to indicate where to place the labware. ☐ Partially full boxes are supported for the Flexible Channel Arm filtered tips. However, please ensure the tip box contains the minimum required number of tips as described in the equipment and consumables section of this protocol. ☐ Click 'Approve' after each addition of labware to proceed to the next box. ☐ After loading all of the required labware, click 'Next Page' to proceed. Follow the on-screen directions to load the metal lid for ODTC on to the worktable: ☐ The required loading position will flash to indicate where to place the labware. ☐ Click 'Approve' after the addition of the metal lid for ODTC to proceed. Follow the on-screen directions to load the Waste plate on to the worktable: ☐ The required loading position will flash to indicate where to place the labware. ☐ After loading the Waste plate, click 'Next Page'.	

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INSTRUCTIONS	NOTES/OBSERVATIONS

Follow the on-screen directions to load the reaction plates onto the worktable:

- ☐ For the reaction plate(s) loaded in the 'Hotel': The lettered well markers (A-H) should be positioned towards the back of the worktable and the numbered well markers (1-12) should be positioned facing the right.
- ☐ For the reaction plate(s) loaded onto the worktable: Follow standard plate orientation, with the lettered well markers (A-H) positioned to the left and the numbered well markers (1-12) positioned to towards the back of the worktable.

  Note: We recommend all plates are labelled before placing on the worktable to ensure correct plate tracking.
- ☐ The required loading position for each plate will flash on the on-screen display to indicate where to place the labware.
- ☐ Click 'Approve' after each addition of labware to proceed.
- ☐ After loading all of the required labware, click 'Next Page'.

Follow the on-screen directions to load the Bead plate, the Elution buffer plate and Ethanol plate on to the worktable:

- ☐ The required loading position will flash to indicate where to place the labware.
- ☐ Click 'Approve' after each addition of labware to proceed.
- ☐ After loading all of the required labware, click 'Next Page'.

☐ Close the front safety shield of the Tecan DreamPrep NGS while you prepare the reaction master mixes off-deck.

Prepare the End-prep (EP) master mix in a 2 ml Sarstedt tube with the following reagents according to the Tecan DreamPrep NGS user interface. Click 'OK' and 'Continue' to proceed.

- ☐ NEBNext FFPE DNA Repair Buffer
  - Volume per sample: 3.5 µl
  - Volume X8 samples: 36.4 µl
  - Volume X24 samples: 109.2 µl
  - Volume X48 samples: 201.6 µl
  - Volume X96 samples: 369.6 µl
- ☐ NEBNext FFPE DNA Repair Mix
  - Volume per sample: 2 µl
  - Volume X8 samples: 20.8 µl
  - Volume X24 samples: 62.4 µl
  - Volume X48 samples: 115.2 µl
  - Volume X96 samples: 211.2 µl
- ☐ Ultra II End-prep reaction buffer
  - Volume per sample: 3.5 µl
  - Volume X8 samples: 36.4 µl
  - Volume X24 samples: 109.2 µl
  - Volume X48 samples: 201.6 µl
  - Volume X96 samples: 369.6 µl
- ☐ Ultra II End-prep enzyme mix
  - Volume per sample: 3 µl
  - Volume X8 samples: 31.2 µl
  - Volume X24 samples: 93.6 µl
  - Volume X48 samples: 172.8 µl
  - Volume X96 samples: 316.8 µl

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INSTRUCTIONS	NOTES/OBSERVATIONS

Prepare the Adapter-ligation (AL) master mix directly into the 2 ml Sarstedt tube(s) with the following reagents according to the Tecan DreamPrep NGS user interface. Click 'OK' and 'Continue' to proceed.

- [ ] Number of tubes to prepare
  - Volume per sample: -
  - Volume X8 samples: 1
  - Volume X24 samples: 1
  - Volume X48 samples: 2
  - Volume X96 samples: 4
- [ ] Ligation Buffer (LNB)
  - Volume per sample: 25 µl
  - Volume X8 samples: 260 µl
  - Volume X24 samples: 780 µl
  - Volume X48 samples: 660 µl
  - Volume X96 samples: 660 µl
- [ ] NEBNext Quick T4 DNA Ligase
  - Volume per sample: 10 µl
  - Volume X8 samples: 104 µl
  - Volume X24 samples: 312 µl
  - Volume X48 samples: 264 µl
  - Volume X96 samples: 264 µl
- [ ] Ligation Adapter (LA)
  - Volume per sample: 5 µl
  - Volume X8 samples: 52 µl
  - Volume X24 samples: 156 µl
  - Volume X48 samples: 132 µl
  - Volume X96 samples: 132 µl

Load the DNA repair and end-prep master mix and Adapter ligation master mix prepared above in the 2 ml Sarstedt tubes into the required positions in the POGO tube holder by following the on-screen instructions.

- [ ] Ensure the master mixes are thoroughly mixed before loading.
- [ ] You will need to select each loading position using the Tecan's TouchTools touchscreen display.
- [ ] Follow the instructions on the display for each reagent, ensuring the fill volume for each tube is correct.
- [ ] Ensure the reagents have been added to all of the required positions before proceeding.
- [ ] Click 'Confirm' to proceed.

IMPORTANT

- [ ] Please ensure the liquid is evenly distributed across the troughs.

IMPORTANT

Depending on the wash buffer (LFB or SFB) used, the clean-up step after adapter ligation is designed to either enrich for DNA fragments of >3 kb, or purify all fragments equally.

- [ ] To enrich for DNA fragments of 3 kb or longer, use Long Fragment Buffer (LFB)
- [ ] To retain DNA fragments of all sizes, use Short Fragment Buffer (SFB)

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INSTRUCTIONS	NOTES/OBSERVATIONS
Load the troughs with their relevant reagents into the worktable by following the on-screen instructions. ☐ Ensure all the reagents have been thoroughly mixed by vortexing before dispensing into the troughs. ☐ The required loading position will flash to indicate where to insert the trough. ☐ Click 'Approve' after each addition to proceed. ☐ After loading all of the troughs, click 'Next Page'. ☐ Site 2: Trough with fresh 80% ethanol. ☐ Site 3: Trough with AMPure XP Beads. ☐ Site 5: Trough with Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB), depending on use. ☐ Site 6: Trough with Elution Buffer (EB). ☐ Site 7: Trough with Nuclease-free water. ☐ Close the front safety shield of the Tecan DreamPrep NGS while you prepare the sample plate off-deck. Follow the on-screen directions to load the sample plate on to the worktable: ☐ Quantify your sample input using a Qubit fluorometer (or equivalent). ☐ Per sample, transfer 1 µg (or 100-200 fmol) of input DNA into a well of the input plate. ☐ Adjust the volume to 60 µl with Nuclease-free water. ☐ Mix thoroughly by pipetting. ☐ Spin down briefly in a microfuge. ☐ The required loading position will flash to indicate where to place the labware. ☐ Click 'Approve' after the addition of labware to proceed. ☐ After loading all of the required labware, click 'Next Page'. ☐ Close the front safety shield of the Tecan DreamPrep NGS before starting your run and click 'Continue'. ☐ Click 'Confirm' and 'Continue' to start the automated library preparation. ☐ Remove the plate containing the eluted libraries from the Tecan DreamPrep NGS deck. ☐ Quantify 1 µl of each eluted sample from the output plate using a Qubit fluorometer plate reader off deck.	
Seal the plate and store on ice until ready to prepare the library/libraries and load onto the flow cell.	
☐ Depending on your required data output, prepare your final library to 35-50 fmol for high output of simplex data, or 10-20 fmol for duplex data, in 12 µl of Elution Buffer (EB).	
Priming and loading the SpotON flow cell	
IMPORTANT ☐ Please note, this kit is only compatible with R10.4.1 flow cells (FLO-MIN114). ☐ Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at RT before mixing by vortexing. Then spin down and store on ice.	

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INSTRUCTIONS	NOTES/OBSERVATIONS

IMPORTANT

- [ ] For optimal sequencing performance and improved output on MinION R10.4.1 flow cells (FLO-MIN114), we recommend adding Bovine Serum Albumin (BSA) to the flow cell priming mix at a final concentration of 0.2 mg/ml.

Prepare the flow cell priming mix with BSA in a suitable vial for the number of flow cells to flush. Once combined, mix well by inverting the tube and pipette mix at RT.

- [ ] 30 µl Flow Cell Tether (FCT)
- [ ] 1170 µl Flow Cell Flush (FCF)
- [ ] 5 µl Bovine Serum Albumin (BSA) at 50 mg/ml

- [ ] Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact.

- [ ] Slide the flow cell priming port cover clockwise to open the priming port.

IMPORTANT

- [ ] 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.

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

- [ ] Set a P1000 pipette to 200 µl
- [ ] Insert the tip into the priming port
- [ ] Turn the wheel until the dial shows 220-230 µl, to draw back 20-30 µl, or until you can see a small volume of buffer entering the pipette tip

  Note: Visually check that there is continuous buffer from the priming port across the sensor array.

- [ ] Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for five minutes. During this time, prepare the library for loading by following the steps below.

- [ ] Thoroughly mix the contents of the Library Beads (LIB) by pipetting.

IMPORTANT

- [ ] The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.

In a new 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:

- [ ] 37.5 µl Sequencing Buffer (SB)
- [ ] 25.5 µl Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), if using
- [ ] 12 µl DNA library

Complete the flow cell priming:

- [ ] Gently lift the SpotON sample port cover to make the SpotON sample port accessible.
- [ ] Load 200 µl of the priming mix into the flow cell priming port (not the SpotON sample port), avoiding the introduction of air bubbles.

- [ ] Mix the prepared library gently by pipetting up and down just prior to loading.

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Add 75 µl of the prepared library to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next. ☐ Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port.	
IMPORTANT ☐ Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.	
Place the light shield onto the flow cell, as follows: ☐ Carefully place the leading edge of the light shield against the clip. Note: Do not force the light shield underneath the clip. ☐ Gently lower the light shield onto the flow cell. The light shield should sit around the SpotON cover, covering the entire top section of the flow cell.	
Close the device lid and set up a sequencing run on MinKNOW.	
Unloading the Tecan DreamPrep NGS worktable	
IMPORTANT ☐ Please ensure you have removed the plate containing your eluted sample libraries and stored it appropriately before unloading the rest of the worktable.	
☐ Empty the tip waste container. ☐ Remove all the remaining plates from the worktable and hotel sites, and discard accordingly. ☐ Remove the disposable 25 ml and 100 ml troughs from the trough mounting sites, and discard accordingly. ☐ Remove the Sarstedt tubes from the POGO tube holder, and discard accordingly. Clean the Bio-Rad™ Arched Auto-Sealing Lid: ☐ Wipe the lid with 10% bleach ☐ Thoroughly rinse the bleach off the lid using ≥80% ethanol or double distilled water (ddH2O) and lint-free wipes ☐ Allow the lid to air dry Remove and/or restock the tip boxes on the worktable: ☐ Remove all Flexible Channel Arm (FCA) hanging tip trays from the FCA standard tip carriers, and discard accordingly. ☐ Remove all empty MultiChannel Arm (MCA) tip boxes from the worktable and discard accordingly. ☐ For partially used tip boxes, consider restacking the box with the appropriate tips for the next run. ☐ In cases where spillage has occurred during the automated library preparation, wipe the worktable surface using ≥80% ethanol.	
Conclude all open dialogues on the TouchTools screen.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Flow cell reuse and returns	
☐ After your sequencing experiment is complete, if you would like to reuse the flow cell, please follow the Flow Cell Wash Kit protocol and store the washed flow cell at 2-8°C. ☐ Alternatively, follow the returns procedure to flush out the flow cell ready to send back to Oxford Nanopore.	
IMPORTANT ☐ If you encounter issues or have questions about your sequencing experiment, please refer to the Troubleshooting Guide that can be found in the online version of this protocol.	