Oxford nanopore sequencing work-flow

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Ligation sequencing kit – basic steps

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1. Quality check of flow cells
2. Repair the DNA and prepare the DNA ends of adapter attachment
3. Clean-up the repaired and end-prepped DNA
4. Attach the sequencing adapters to the DNA ends
5. Clean-up the DNA attached adapter
6. Prepare flow cell for sequencing
7. Prepare DNA library
8. Set up a sequencing run (MinKNOW software)

DNA input

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- HMW of DNA: 1 ug (or 100 ~ 200 fmol)
  (if performing DNA fragmentation, MW of ≥ 100 ng)

- Input volume is 47 μl, which works out to be ≒ 21.3 ng/μl

1. Quality check the flow cell

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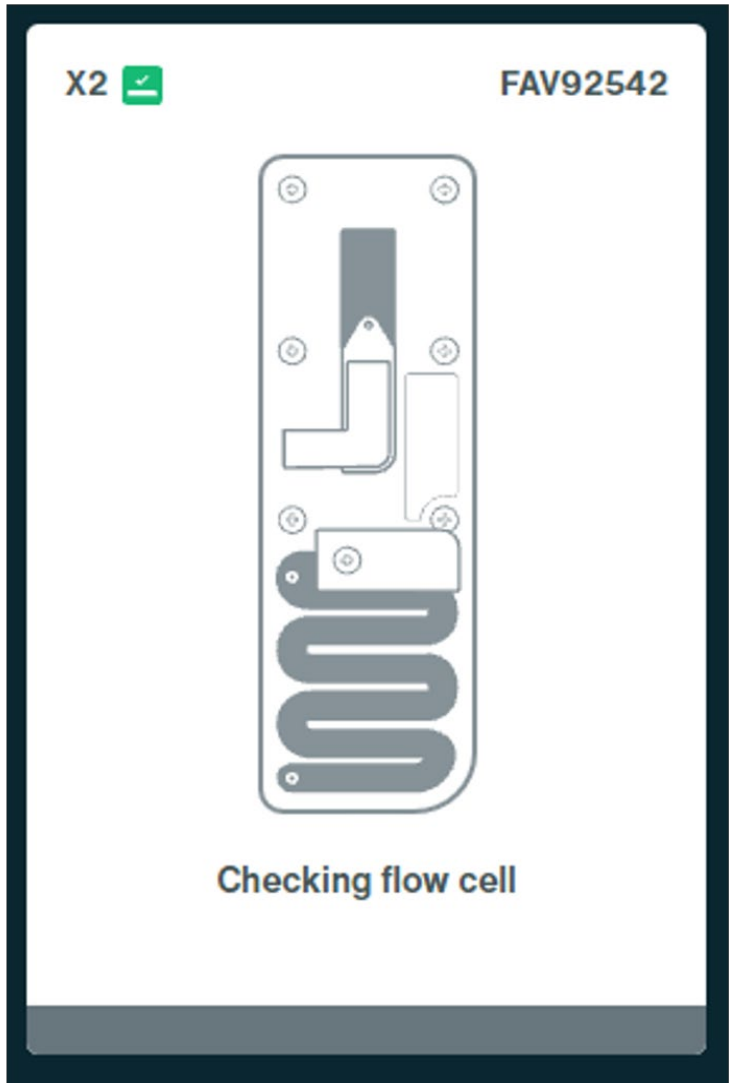
- Quality check (QC) of flow cells should be done within three month of them arriving in your laboratory
- **If MinION flow cells are below the warranty of 800 pores, user can request replacements!!**
- Only need to QC once, MinKNOW will automatically QC again when a sequencing run is started.
- It will identify how many active pores user have on users' flow cell.
- The more pores that are active, the more data should obtain.



Processing quality check the flow cell

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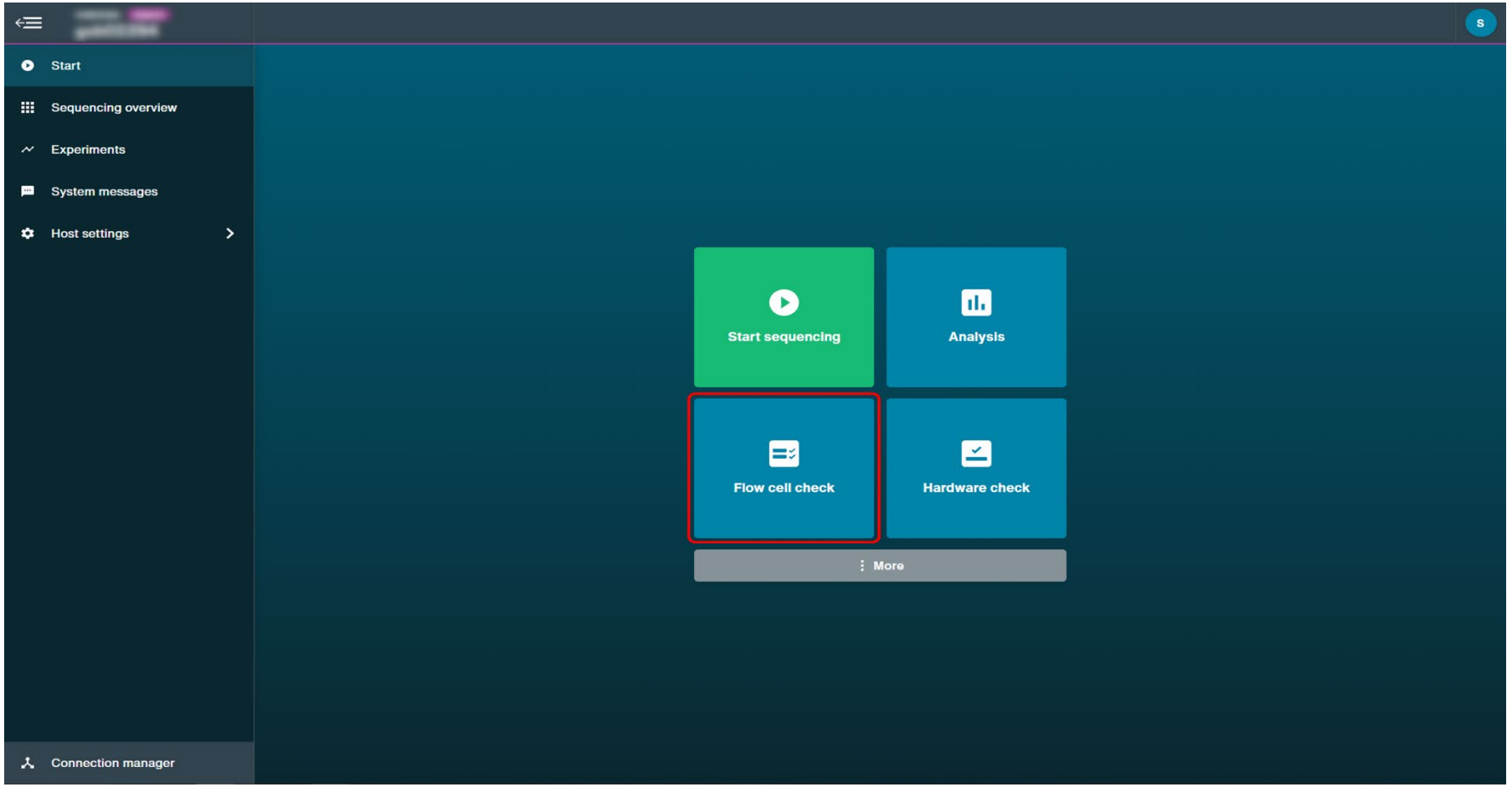
1. Navigate to the start homepage and click ‘Flow cell check’



Processing quality check the flow cell

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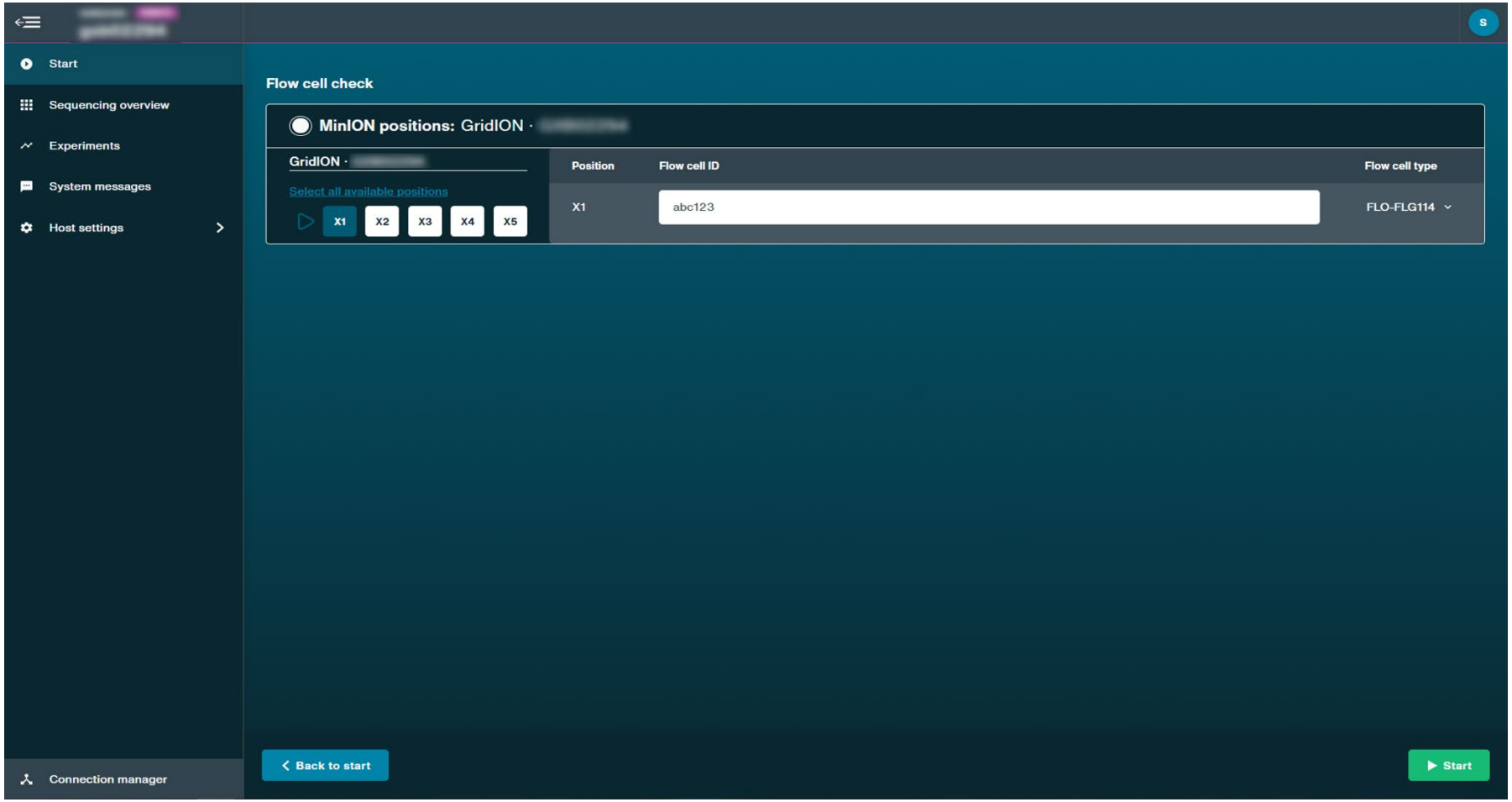
2. When you see the flow cell type and flow cell ID is recognized, click ‘Start’ to begin.

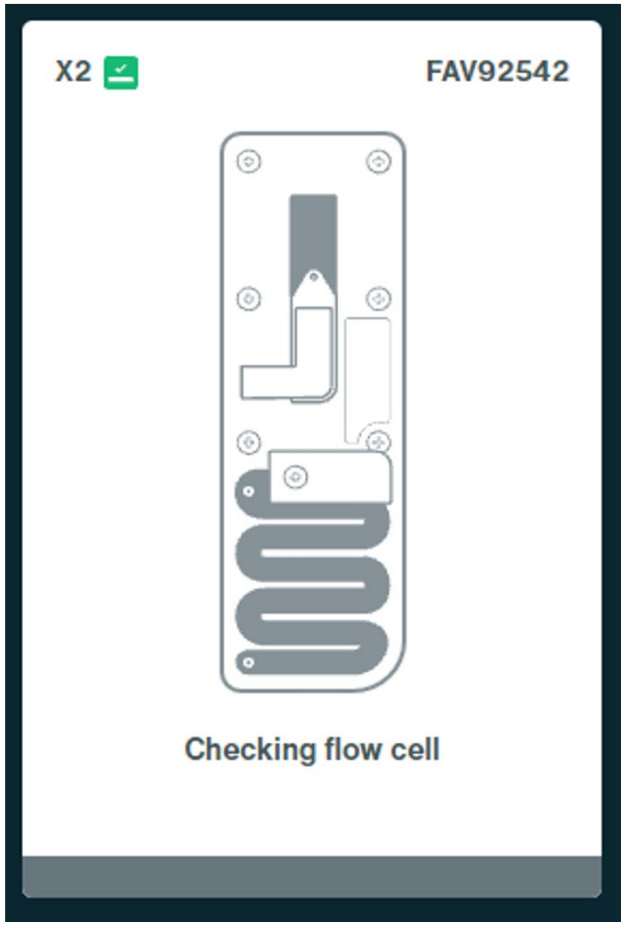


ex) On a GridION device

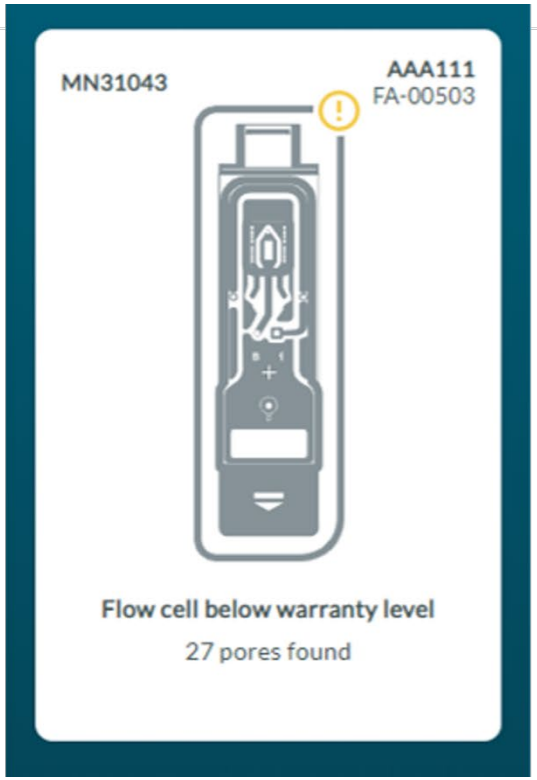
For Flongle, the flow cell ID must be filled in manually.

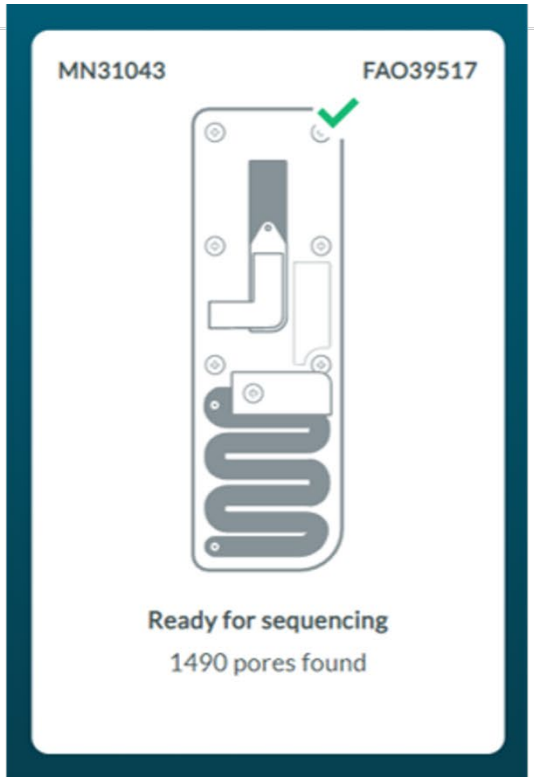
Processing quality check the flow cell

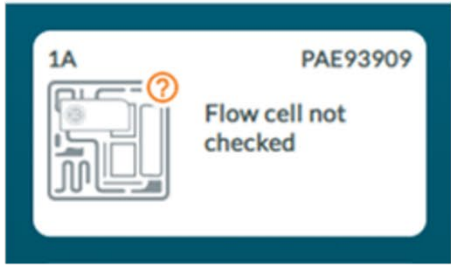
3. Automatically navigated to the Sequencing Overview page.



Flow cell health indicators







Mark	Status of sequencing pores
Yellow exclamation (!)	The number of sequencing pores is below warranty
Green tick (v)	The number of sequencing pores is above warranty and ready to sequencing
Question (?)	A flow cell check has not been run on the flow cell during this MinKNOW session

Ligation sequencing kit (SQK-LSK114)

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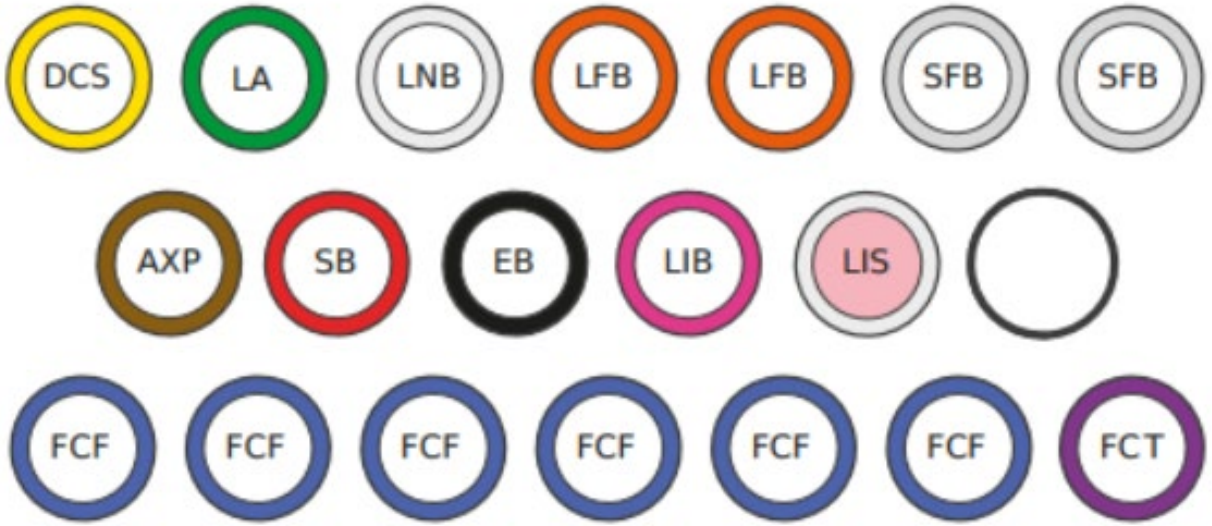
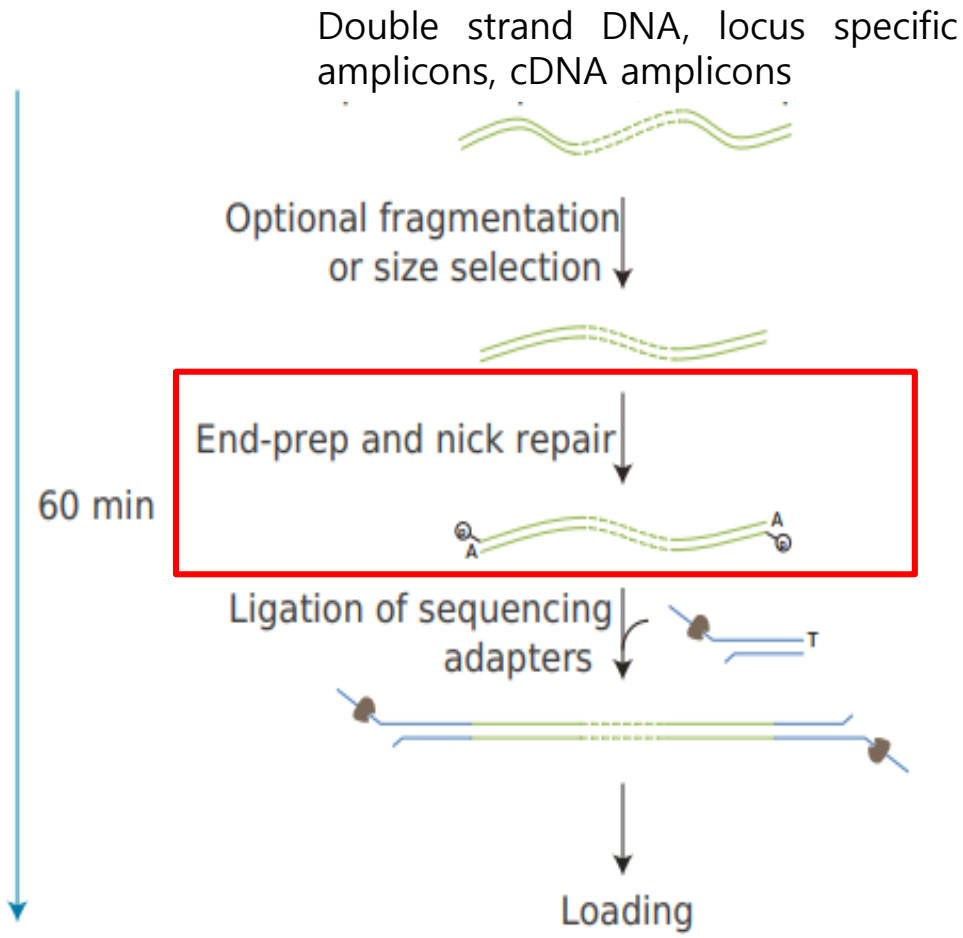


Label	Full name
DCS	DNA Control Strand
LA	Ligation Adapter
LNB	Ligation Buffer
LFB	Long Fragment Buffer
SFB	Short Fragment Buffer
AXP	AMPure XP Beads
SB	Sequencing Buffer
EB	Elution Buffer
LIB	Library Bead
LIS	Library Solution
FCF	Flow Cell Flush
FCT	Flow Cell Tether

1. Repair the DNA and prepare the DNA ends of adapter attachment



1. Ligation sequencing kit reagents

DCS

- Using for troubleshooting purposes.

AXP

- Collecting DNA sample and clean-up

2. NEBNext Companion Module (MEB, E7180S or E7180L)

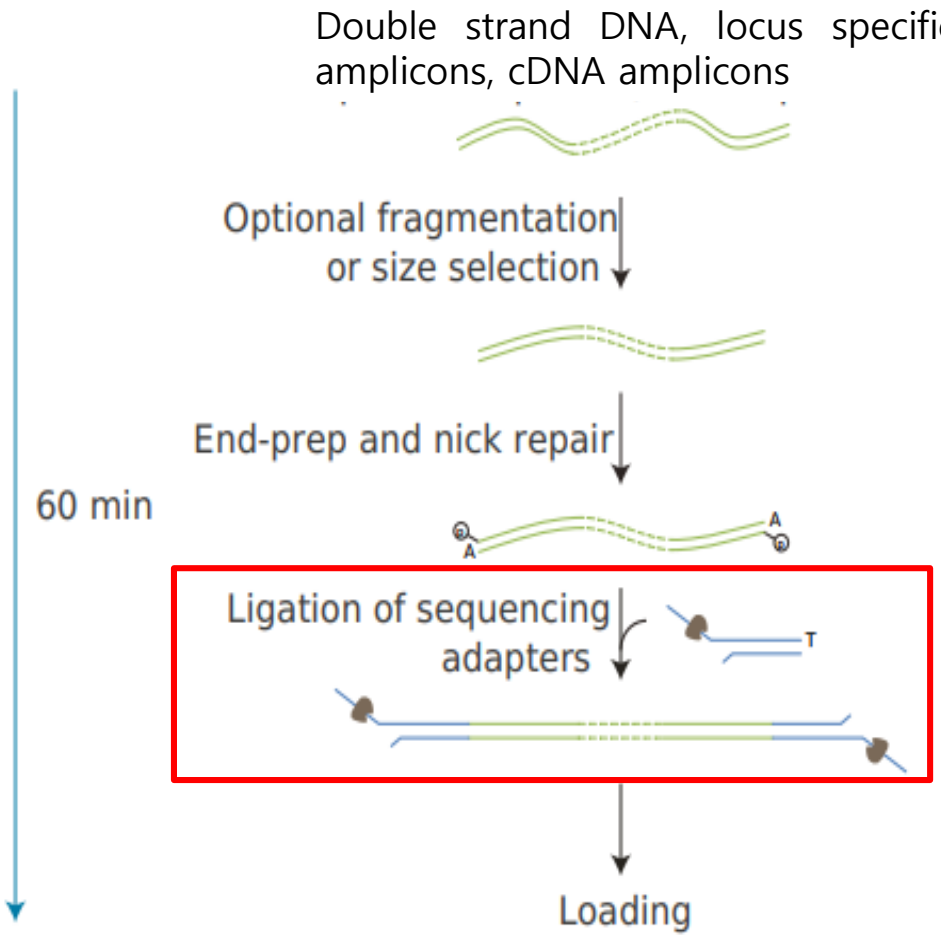
- Using the DNA nick and End-repair

2. Clean-up the repaired and end-prepped DNA

- Once the DNA have been repaired and end-prepped, DNA bind to the beads, and the beads stick to a magnet, enabling removal of supernatant and washing of DNA with 80% ethanol.

- DNA can than be eluted into 61 ul of buffer for adapter attachment.

3. Adapter ligation and clean-up



1. Ligation sequencing kit reagents

Label	Full name
LA	Ligation Adapter
LNB	Ligation Buffer
LFB	Long Fragment Buffer
SFB	Short Fragment Buffer
AXP	AMPure XP Beads
EB	Elution Buffer

2. NEBNext Quick Ligation Module (NEB, E6056)

Steps of adapter ligation and clean-up

1. DNA fragmentation

- Long Fragment Buffer (LFB) → ≥ 3 kb of DNA fragments
- Short Fragment Buffer (SFB) → All size of DNA fragments

2. Adapter Ligation

3. Clean-up the DNA attached adapter

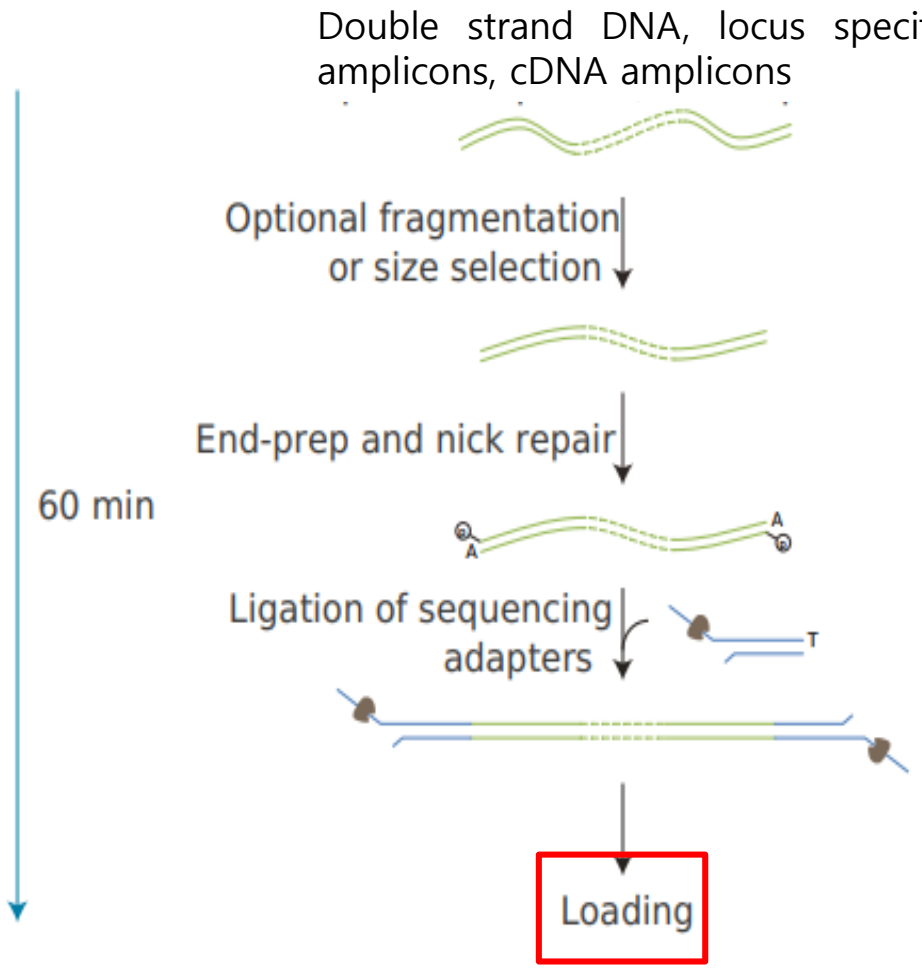
- Once the DNA have been repaired and end-prepped, DNA bind to the beads, and the beads stick to a magnet, enabling removal of supernatant and washing of DNA with 80% ethanol.

- The DNA attached adapter can than be eluted into 15 ul of final library.

- **For high output of simplex data, load 35~50 fmol of final library.**

- **For duplex data, load 10~20 fmol of final library**

4. Priming and loading the MinION and GridION Flow cell



1. Ligation sequencing kit reagents

Label	Full name
LIB	Library Bead
LIS	Library Solution
FCF	Flow Cell Flush
FCT	Flow Cell Tether

Recommend using Library beads (LIB) for most sequencing However, the Library solution (LIS) is available for more viscous libraries

2. MinION and GridION Flow cell

1. Preparation of the flow cell

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- Prepare flow cell – changing storage buffer to sequencing flush buffer
- Flow cells are supplied with storge buffer (yellow in color) covering the active pores.
- The storage buffer must be replaced by sequencing flush buffer (clear in color) to enable the correct conditions for sequencing
- Sequencing flush buffer is made from Flush buffer (FB) and Flush tether (FT).
- **It is VERY IMPORTANT that do not introduce air bubbles into the flow cell when you add the sequencing flush buffer (if air touches a pore, it will become inactive!!)**

Reagent	Volume per flow cell
Flow Cell Flush (FCF)	1,170 µl
Bovine Serum Albumin (BSA) at 50 mg/ml	5 µl
Flow Cell Tether (FCT)	30 µl
Total volume	**1,205 µl**

Flush Buffer: where does it go?

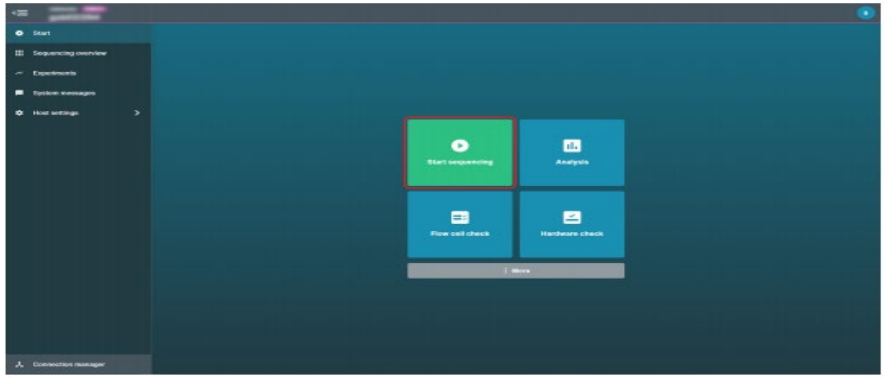
2. Preparation of DNA library

- The DNA attached adapter is then mixed with sequencing buffer (SQB), loading beads (LB) and nuclease free water.
- This mixture is loaded into the SpotON port on the flow cells (approximately, 75 uL)

5. Setting uup a sequencing run

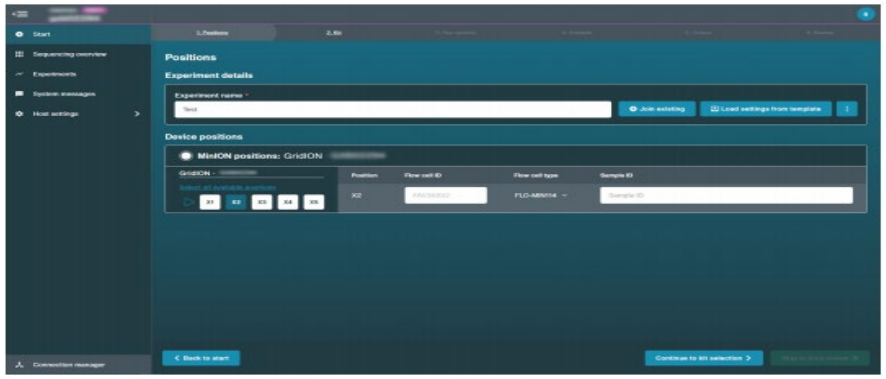
- Once the flow cell has been prepared and the sample loaded, the ports are closed and MinKNOW used to setup a sequencing run.

1



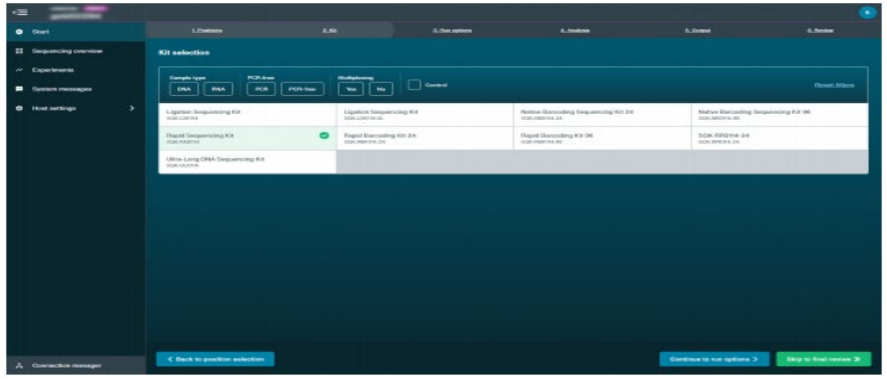
Click Start sequencing

2



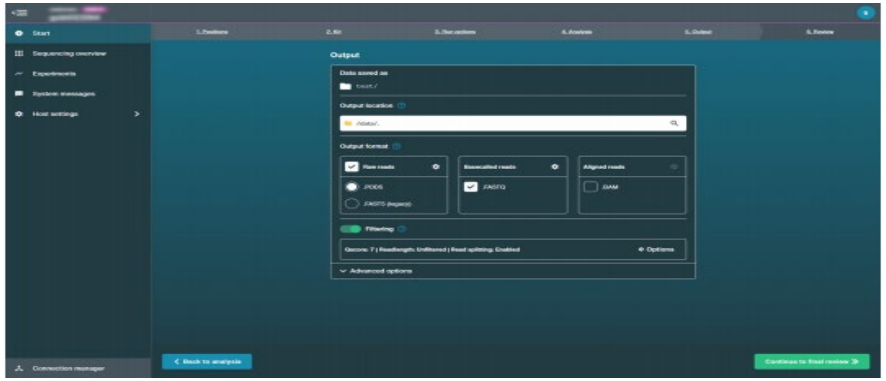
Fill in experiment details (flow cell position, sample ID)

3



Select the sequencing kit used

4



Set up the output parameters