Introduction to the Rapid Barcoding Kit (SQK-RBK110.96)

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We will record these sessions and put them online so you can refer back to them later on

We will also put the slides up online so you can access the notes (links and image credits)

Why the Rapid Barcoding Kit (SQK-RBK110.96)?

- Quick and user friendly
- Can multiplex up to 96 samples per flow cell run (~12-24 for whole genome samples)
- Relatively good output (for long reads to be observed in sequencing, long fragments need to be present in the sample in the first place)
- Better coverage compared to PCR based kits (and therefore removes PCR bias – see link in notes)
- Cheaper than other kits (e.g. ligation kit) as no other third party reagents are needed

Rapid Barcoding Kit – basic steps

1. QC flow cell
2. Add barcodes to each sample
3. Bead wash to reduce library volume
4. Attach rapid sequencing adaptor protein
5. Prepare flow cell for sequencing
6. Prepare DNA library
7. Set up a sequencing run (MinKNOW software)

Equipment and consumables needed for Rapid Barcoding Kit

- Magnetic bead rack
- Thermal cycler at 30°C and 80°C
- P1000 pipette and tips
- P100 pipette and tips
- P20 pipette and tips
- P10 pipette and tips
- P2 pipette and tips
- 80% ethanol
- Nuclease free water
- PCR tubes
- 1.5 mL Lo-bind tubes
- Timer
- Microfuge
- Vortex mixer
- 4°C fridge
- -20°C Freezer

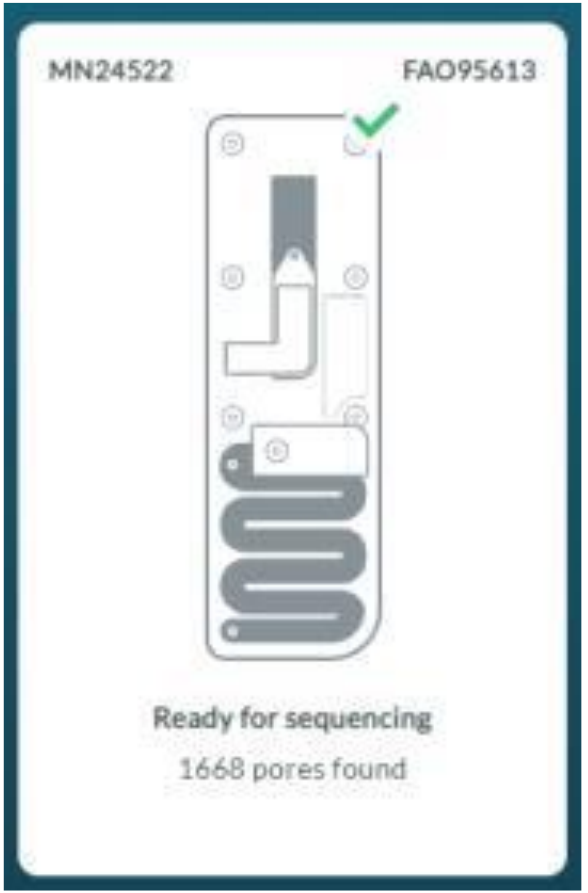
DNA input

- 50-400 ng and MW of >30kb*
- Input volume is 9 µL, which works out to be ~5-44 ng/µL
- If your samples have very different MWs, try normalising for fmol instead to get more even numbers of reads per barcode (see notes below for online calculator)

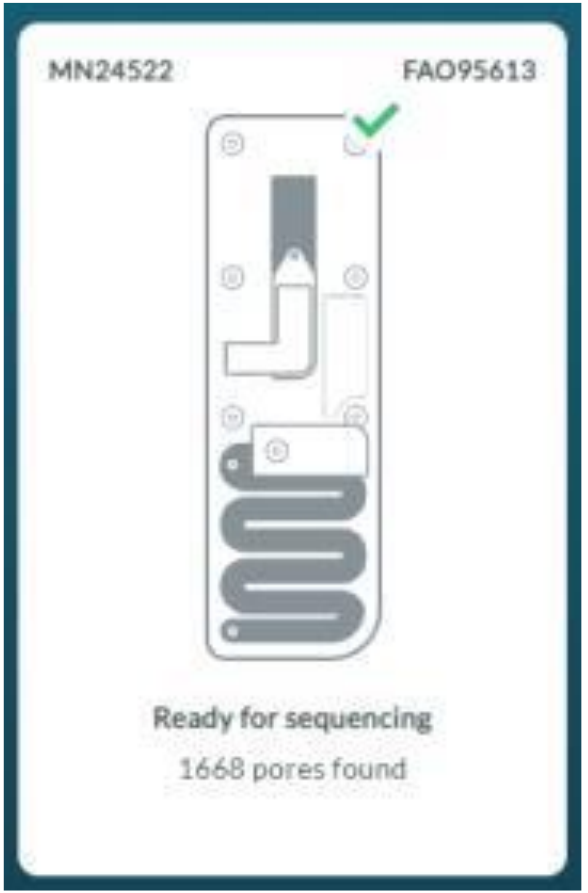
* For tuberculosis = may want lower MW (trade off between long reads and pore efficiency, see previous presentation)

1. QC the flow cell

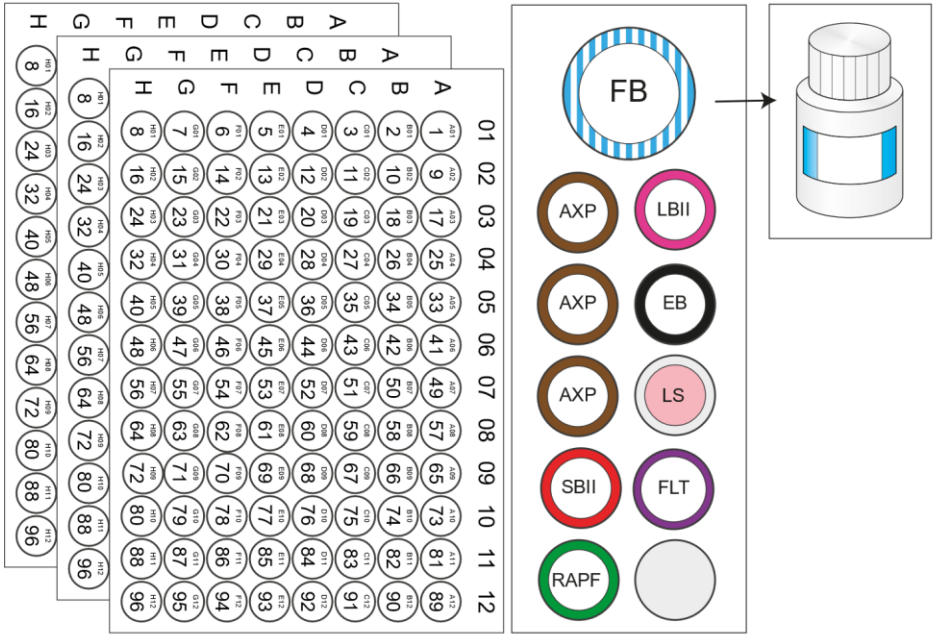
- Quality Control (QC) of flow cells should be done within a few days of them arriving in your laboratory (if they are below the warranty of 800 pores, you can request replacements)
- You only need to QC once, MinKNOW will automatically QC again when a sequencing run is started
- This will identify how many active pores you have on your flow cell (the more pores that are active, the more data you should obtain)



1. QC the flow cell



Rapid barcoding 96 kit: What's in the box?

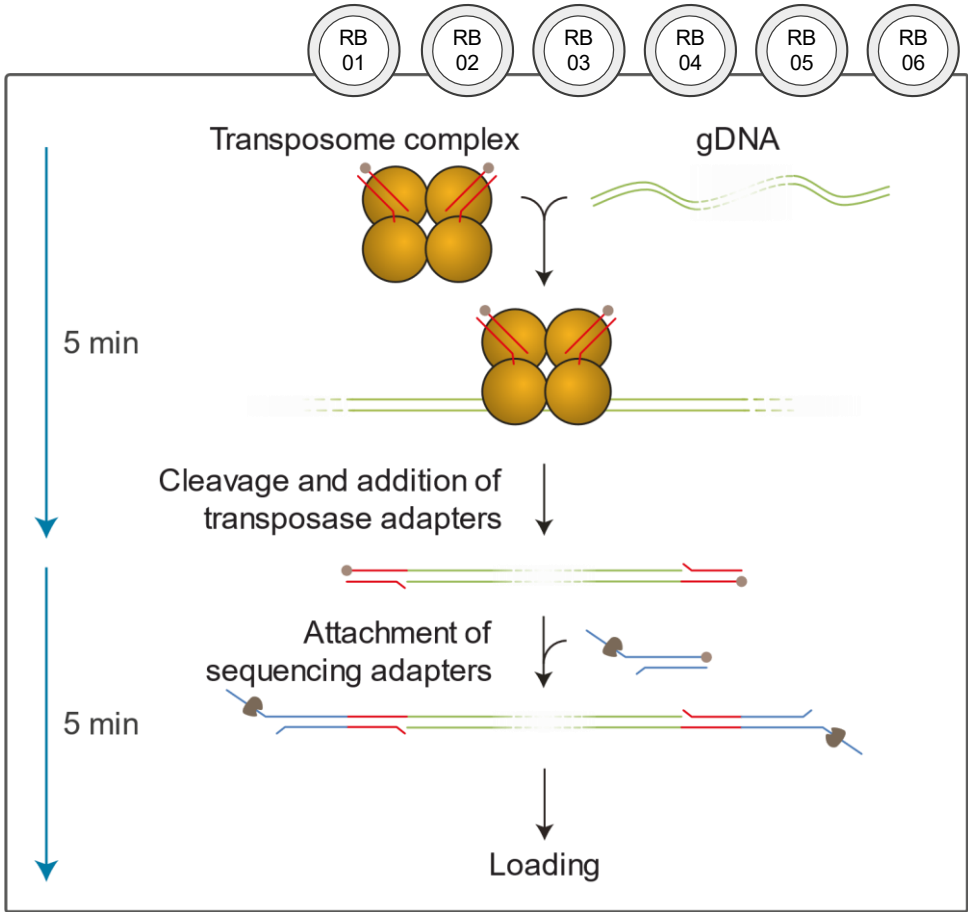


FB : Flush Buffer
AXP : AMPure XPBeads
SBII : Sequencing Buffer II
RAPF : Rapid Adapter F

LBII : Loading Beads II
EB : Elution Buffer
LS : Loading Solution
FLT : Flush Tether

2. Addition of barcodes to samples

- Transposon-based fragmentation: transposase simultaneously cleaves template molecules and attaches barcoded tags to the cleaved ends
- There are 12 unique barcoded tags in the kit
- Barcodes are attached to the DNA by heating
- Once the barcodes are attached, samples can be pooled
- Samples are identified (demultimplexed) by computer programmes such as Guppy)



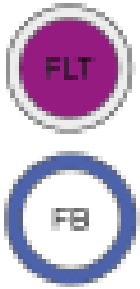
3. Bead wash

- Once the barcodes have been added, the volume needs to be reduced using magnetic beads (can have up to 96 x 9ul)
- DNA binds to the beads, and the beads stick to a magnet, enabling removal of supernatant and washing of DNA with alcohol
- DNA can then be eluted into smaller volume of buffer for library prep

4. Rapid adaptor protein addition

- Once the samples are pooled, the **Rapid Adaptor Protein (RAP)** is added (incubation for 5 minutes at room temperature)
- RAP helps to guide the DNA to the pores

5. Preparation of the flow cell



- Prepare flow cell – changing storage buffer to sequencing flush buffer
- Flow cells are supplied with storage buffer (yellow in colour) covering the active pores
- The storage buffer must be replaced by **sequencing flush buffer** (clear in colour) to enable the correct conditions for sequencing
- **Sequencing flush buffer** is made up from **Flush buffer (FB)** (1170 µL) and **Flush tether (FT)** (30 µL) (both come in the **Flow Cell Priming Kit**)
- The Flow Cell Priming Kit comes with each sequencing preparation kit
- It is VERY IMPORTANT that you do not introduce air bubbles into the flow cell when you add the sequencing flush buffer (if air touches an pore, it will become inactive)

Flush Buffer: where does it go?

6. Preparation of DNA library

- The barcoded DNA (with attached RAP) 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 cell (approx. 75 µL)

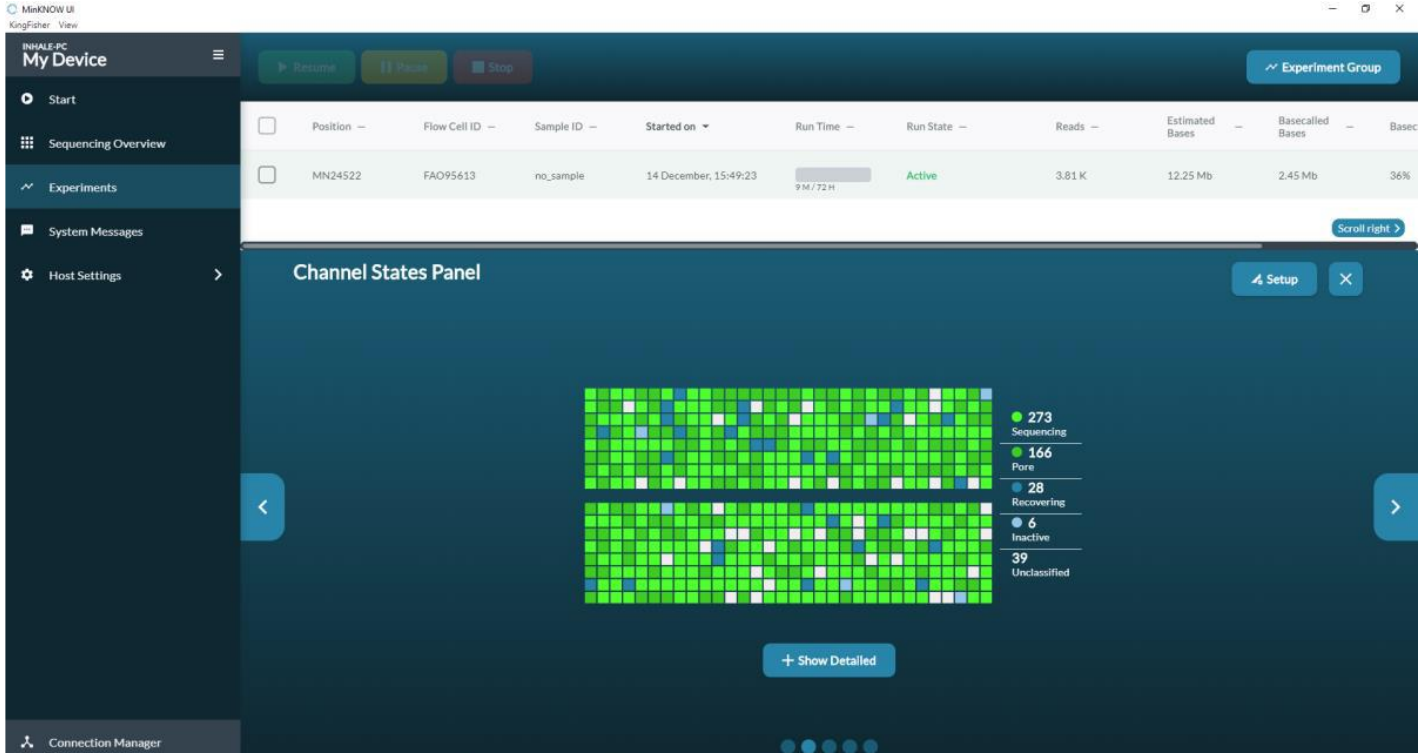
Sample loading: where does it go?

Sample loading: where does it go?

7. Setting up a sequencing run

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

7. Setting up a sequencing run



Task:

You know that you need 50-400 ng of DNA in 9 µL

50-400 ÷ 9 = 5.5-44.4 ng/µL

You now need to work out how many µL of each sample to input to ensure you have the correct ng of each sample

And work out how much water you need to add to make up 9 µL

Concentration calculator (C1V1=C2V2)

C1 = starting concentration
V1 = starting volume
C2 = final concentration
V2 = final volume

Concentration calculator (C1V1=C2V2)

C1 = Different for each sample
V1 = ?
C2 = 5-44 ng/ul
V2 = 9 ul

Concentration calculator (C1V1=C2V2)

$$V1 = V2 * C2 / C1$$

Sequencing

C2 = (G1=7.1, G2=17.2 and G3=44)

V2 = 9

V1 = V2 * C2 / C1

Don’t forget to calculate the water!

sample	ng/ul	group
93385	7.1	1
92130	7.84	
91023	10.9	
80637	10.9	
91993	13.2	
91764	13.2	
92593	17.2	2
93859	17.4	
91071	20	
90623	42.4	
91420	42.8	
90805	44	
92201	56.2	3
93002	56.4	
90417	60.4	
91545	75.8	
93620	110	
93101	114	

Amount of sample DNA needed:

sample	ng/ul	group	Normalise to	DNA	NFW	ng
93385	7.1	1	7.1	9.0	0.0	63.9
92130	7.84			8.2	0.8	
91023	10.9			5.9	3.1	
80637	10.9			5.9	3.1	
91993	13.2			4.8	4.2	
91764	13.2			4.8	4.2	
92593	17.2	2	17.2	9.0	0.0	154.8
93859	17.4			8.9	0.1	
91071	20			7.7	1.3	
90623	42.4			3.7	5.3	
91420	42.8			3.6	5.4	
90805	44			3.5	5.5	
92201	56.2	3	44	7.0	2.0	396
93002	56.4			7.0	2.0	
90417	60.4			6.6	2.4	
91545	75.8			5.2	3.8	
93620	110			3.6	5.4	
93101	114			3.5	5.5	