Centre for Clinical Microbiology

Practical considerations for setting up Nanopore sequencing

Dr Linzy Elton

Postdoctoral research associate

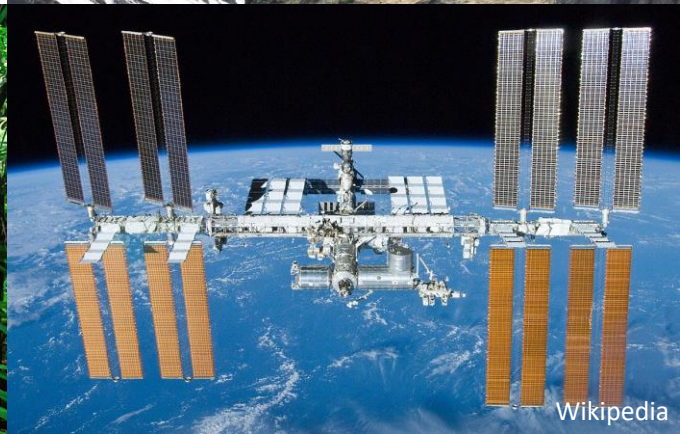
- Introduction
- Lead times
- Reagents – storage
- Flow cells
- Sample throughput
- Data storage systems
- Internet access

Introduction

- Nanopore technology (Flongle and MinION, VolTRAX and MinIT) are designed to be used in both the laboratory and the field

Practical implications for setting up Nanopore sequencing

- To enable the analysis of any living thing, by any person, in any environment
- Shown to work in -5°C in Antarctica
- Kabobo, Democratic Republic of Congo – biodiversity (high humidity, equatorial conditions)
- Study of geothermal microbes in Iceland sequenced in a tent using only solar power (entirely ‘off-grid’)

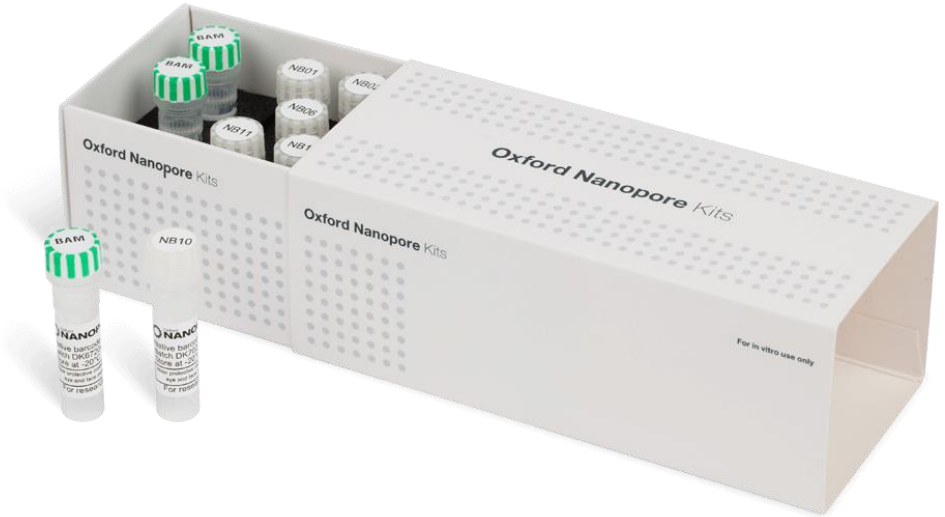


Lead and delivery times

- Some kits and equipment have a lead time (there was a backlog of Flongles), check Nanopore's website before planning your experiments
- Generally, the Nanopore website suggests allowing a working week for orders to arrive for 'rest of world'
- Consider customs issues?

Reagents – Storage

- Some of the kit reagents need a cold chain and so must be kept in a fridge/freezer
- Nanopore have created a field sequencing kit



Field Sequencing Kit

- The kit does not require cold packaging for transportation, or refrigeration when used within a month
- Enables library preparation from genomic DNA in only ten minutes
- It contains lyophilised sequencing chemistry and needs minimal laboratory equipment to prepare a library, with users having successfully prepared samples with a pipette and a cup of hot coffee
- The kit can be stored for up to one month at up to 30°C unopened, or at 2-8° C for three months unopened, and matches the newly released and improved packaging of MinION flow cells

Flow cells

- MinION flow cells can be stored (unopened) at room temperature for 4 weeks (from delivery date)
- They can be stored for 12 weeks (unopened) at 2-8°C
- Flow cells should be checked before library preparation (within 3 months of purchase) as part of the warranty

Sample Throughput

- If few samples are being processed at a time, or you are looking at amplicons, smaller genomes or target regions, the Flongle might be more useful
- They are only guaranteed for 4 weeks after the shipment date (rather than 12 for a MinION)

Power issues

- You will need power for the duration of your sequencing run (if it cuts out, it will save data up until the power cut)
- A laptop with a reliable battery may be more useful than a computer if power cuts are an issue

Power issues

- Having an Uninterrupted power supply (UPS) would be useful
- The MinIT, whilst not needing the internet to sequence, does require an external power source (mains or battery)(data will be saved up until loss of power), so you must consider the reliability of both

Internet Access

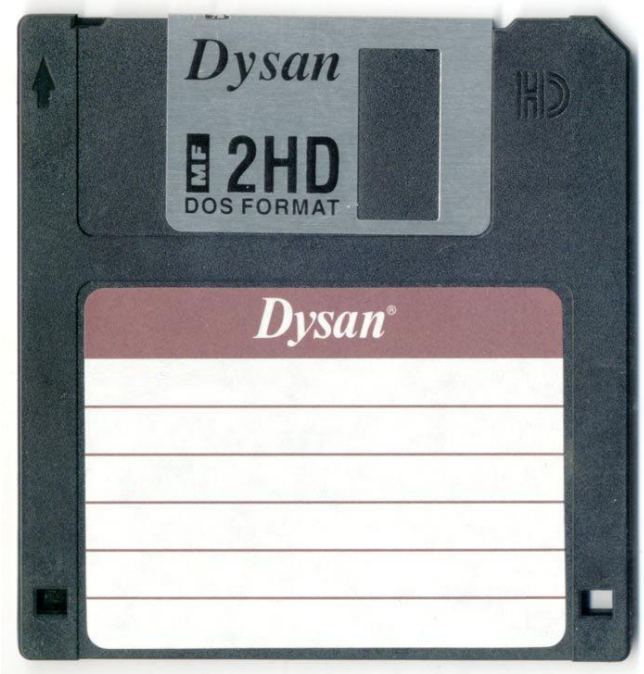
- The MinIT can sequence and basecall entirely without internet connectivity
- To basecall your sequence in real time, you will need internet access for the duration of your MinION reading (MinKNOW uses a cloud-based platform)
- For large sequence runs, it can take longer to basecall

Internet Access

- However, if time isn't an issue, then base-calling can be done separately. You also require internet access to run sequences through TBprofiler
- Offline configurations can be made available for field use and expeditions

Data storage

- A MinION flow cell can generate as much as 30 Gb of data, so storage may be an issue
- Running out of disk space will halt your sequencing experiment
- Users operating the MinION on a lower specification machine (e.g. laptop) will find that MinKNOW will basecall as much as possible in real time and then analyse the remaining files at the end of the sequencing experiment; the laptop will need to be left on for this to happen
- The MinIT has 512 GB SSD storage and provides an average capacity of roughly 30 Gb



Data storage considerations

- Laboratories must consider the general data protection regulations (GDPR) of their data, especially when sequencing directly from a human sample
- Whilst human DNA depletion must be done, ethics surrounding the storage of human sequences needs to be considered)

Summary

- Lead times – one working week delivery to ‘rest of the world’
- Reagents – many reagents require a cold chain, but Nanopore have produced a field kit of lyophilised reagents
- Flow cells – stored unopened for 4 weeks at room temperature and 12 weeks at 2-8°C
- Sample throughput – MinION can run up to 12 samples at a time, Flongle can be used for smaller runs or shorter sequences
- Power – a laptop may be more useful if power supply is unreliable
- Internet access – sequencing must be started with internet access, but can continue without internet access
- Data storage systems – sequencing produces large amounts of data (raw data vs fastq file storage)