Exeter Sequencing Service

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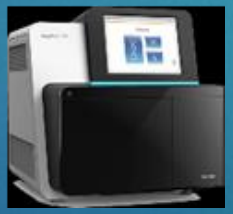
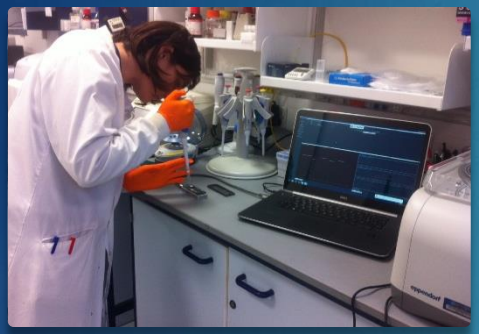
We provide:

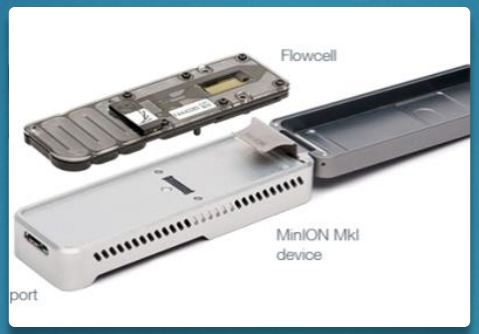
- State of the art genomics and bioinformatics analysis
- Training in experimental techniques, analysis and modelling
- Customised sequencing for researchers in academia and industry
- We support grants worth over £10 million each year
- Annual turnover of around £0.5M.



Oxford Nanopore MinIon Sequencing

Exeter Sequencing Service





Laptop £800

Disable automatic software updates and sleep modes

Software-MinKNOW, Metrichor, MinoTour, Chronolapse

Available storage space >150 Gb

MinIon £1000

- The Minion holds a disposable flow cell and links to a laptop through a USB port
- The flow cell comprises an array of independent sensors that each contains a nanopore

Reliable internet connection

Major advances

- Scientific

Allows charged polymers (such as single-stranded DNA, double-stranded DNA and RNA) to be analysed with sub-nanometre resolution

No requirement for fluorescent labels

No need for amplification.

- Usability

Low initial outlay

Portability

- Business

Open development model

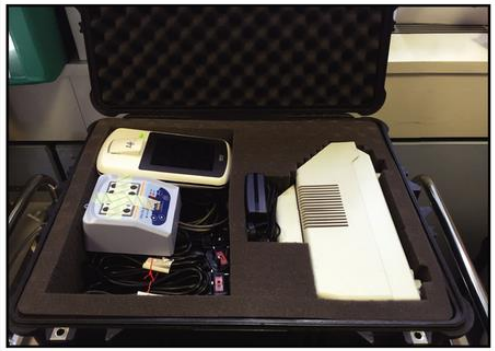
Community driven

Equipment, reagents and consumables

Joshua Quick,
Nicholas J. Loman *et al.*
Nature 530, 228–232
(11 February 2016)

A. Equipment

Item	Number	Model
Thermocycler	1-3	MasterCycler Personal (Eppendorf)
Fluorometer	1	Qubit 3.0 (Life Technologies)
Laptop	2-3	NT310-H (Stone)
MinION	2-3	-
Pipettes	6	P2, 10, 20, 100, 200, 1000 (Gilson)
Microfuge	1-2	
Dry bath	1	Mini Dry Bath Incubator (Starlab)
Magnetic rack	1	MagnaRack (Life Technologies)
Power strip	1	Dependent on country



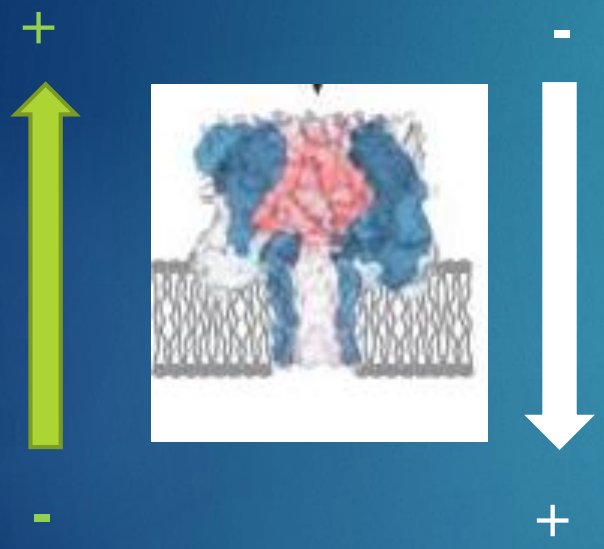
B. Consumables

Item	Supplier
DNA LoBind Tubes (2 ml)	Eppendorf
Protein LoBind Tubes (2 ml)	Eppendorf
Qubit Assay Tubes	Life Technologies
PCR Tubes with Flat Caps (0.2 ml)	Starlab
Pipette Tips (10 µl, 20 µl, 100 µl, 200 µl, 1000 µl)	Sarstedt
Nitrile Gloves	Kimberly Clark Professional

C. Reagents

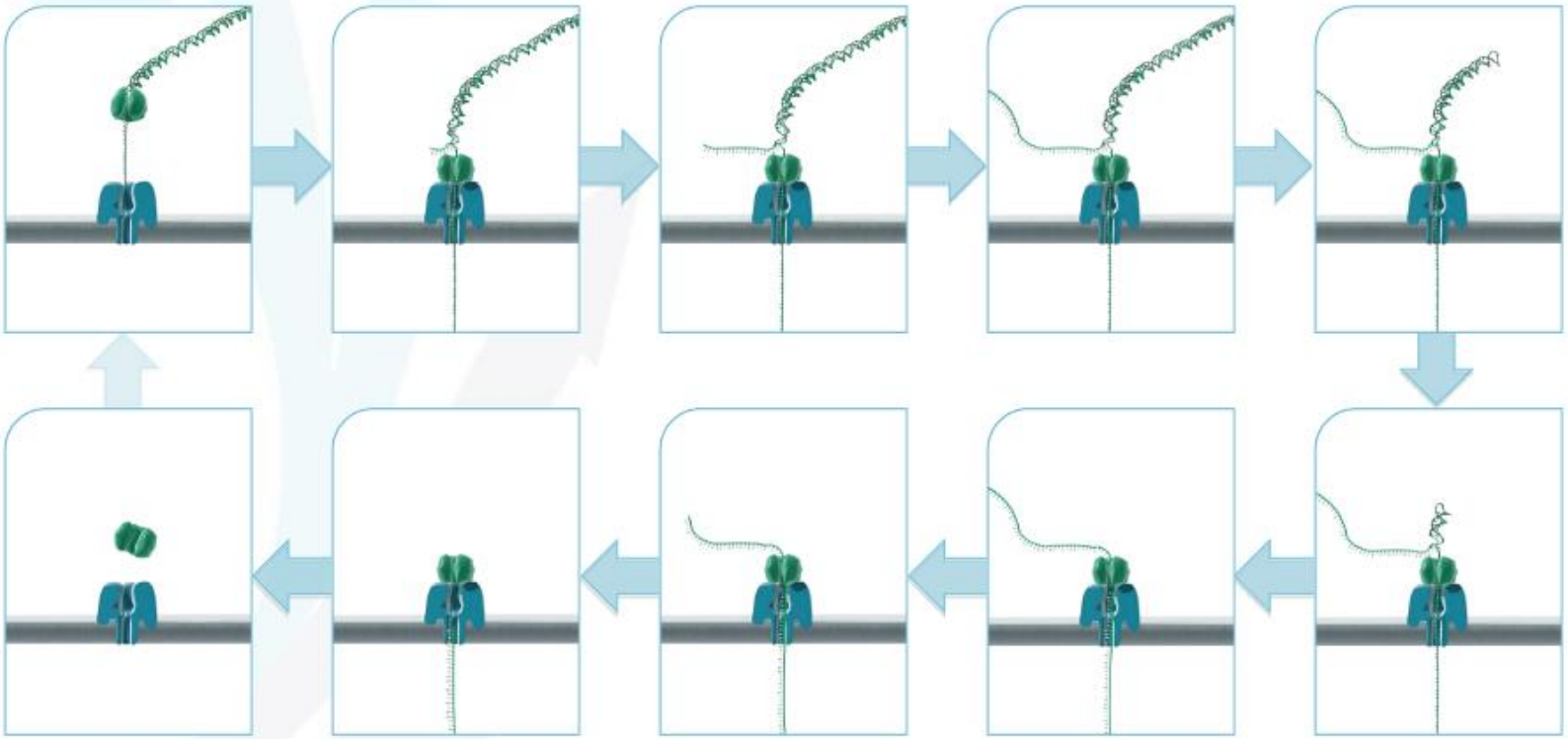
Reagent	Shipping Condition	Supplier
Nuclease-Free Water	Ambient	Qiagen
Ethanol 100%	Ambient	-
HighPrep PCR	Chilled	MAGBIO
Dynabeads His-Tag Isolation and Pulldown	Chilled	Life Technologies
Oligos	Chilled	Sigma
Qubit dsDNA HS Assay Kit	Chilled	Life Technologies
MinION Flowcells	Chilled	Oxford Nanopore Technologies
NEBNext End-Repair Module	Frozen	New England Biolabs
NEBNext dA-Tailing Module	Frozen	New England Biolabs
Blunt/TA Ligase Master Mix	Frozen	New England Biolabs
SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase	Frozen	Life Technologies
SQK-MAP005	Frozen	Oxford Nanopore Technologies

The heart of the matter: Nanopores



- Nanopores are nanoscale pores embedded in an electrically resistant polymer membrane
- An Application Specific Integrated Circuit controls and measures individual channels corresponding to single nanopores
- A voltage across the membrane sets up an ionic current within the nanopores

Sequencing in real time



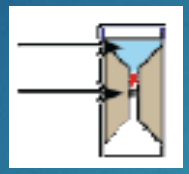
- Proprietary enzyme feeds the template and complement through custom nanopore
- Truly “free-running” system – enzymes self load and strand exits automatically

Oxford NANOPORE Technologies®

Library preparation

High molecular weight DNA

DNA sample constriction



+

G-tube fragmentation
Centrifuge ~2 minutes

End repair and dA-tailing

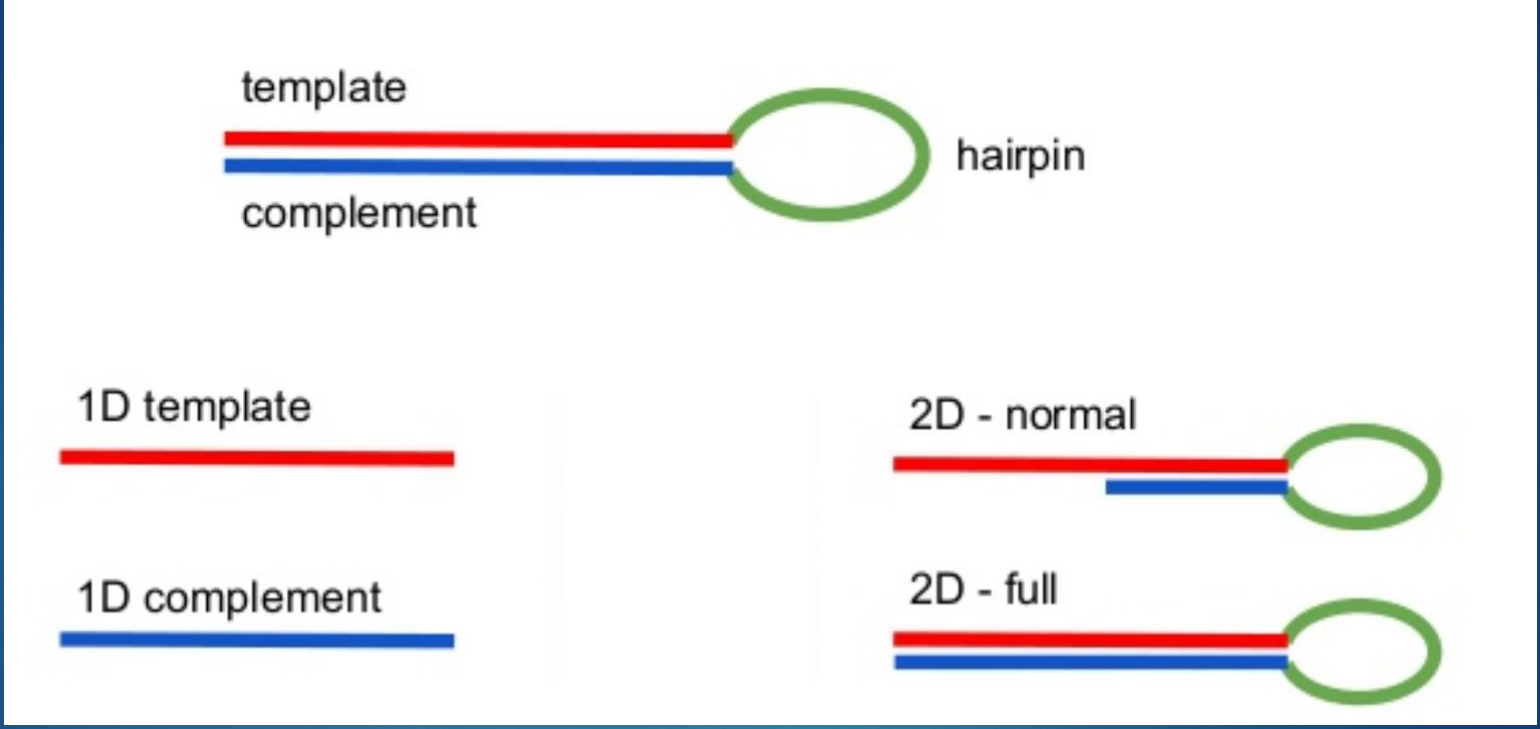
Adapter ligation

Y-shaped adapter with tether

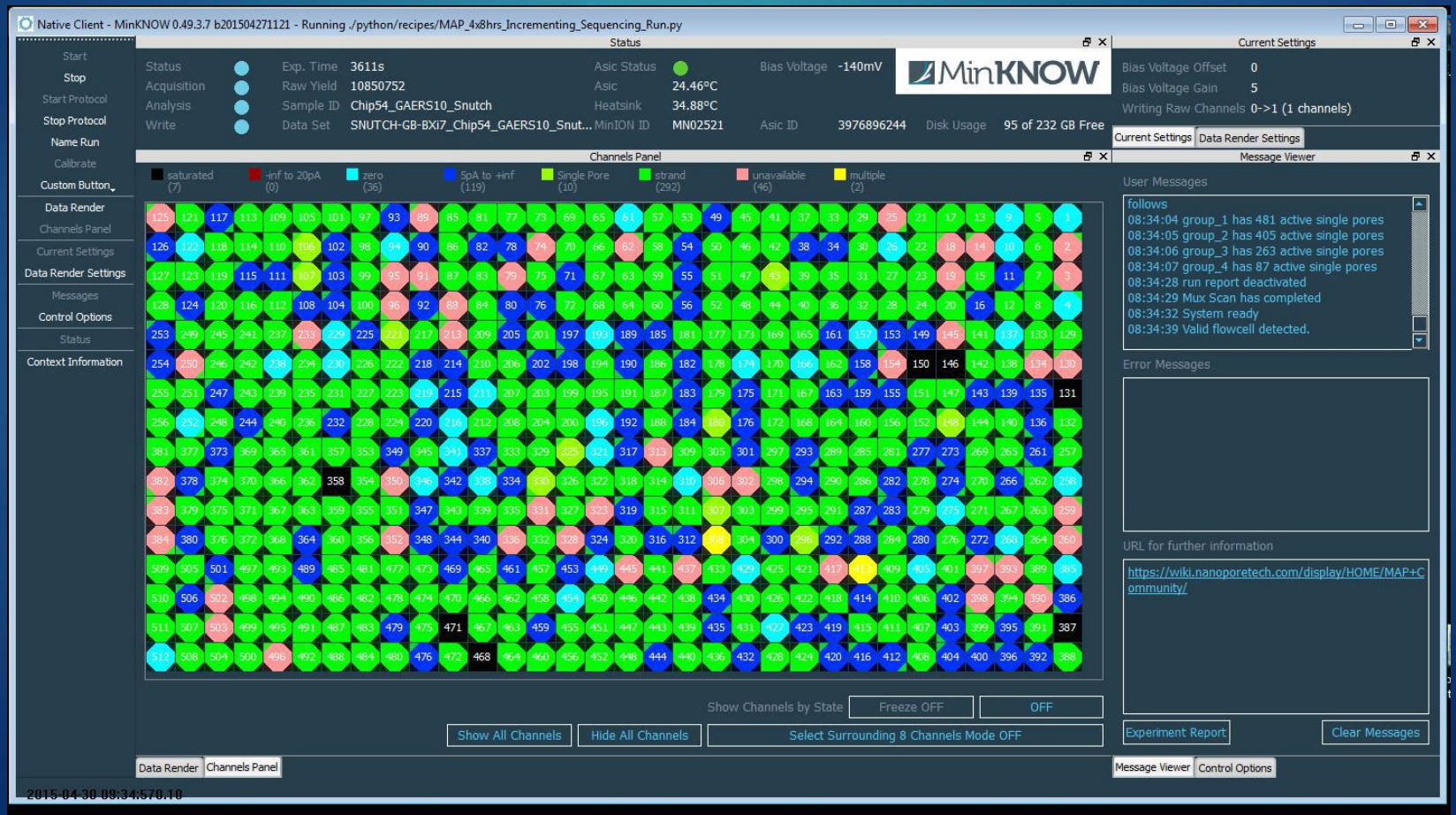
hairpin adapter

molecular motor facilitates active DNA transition through the nanopore

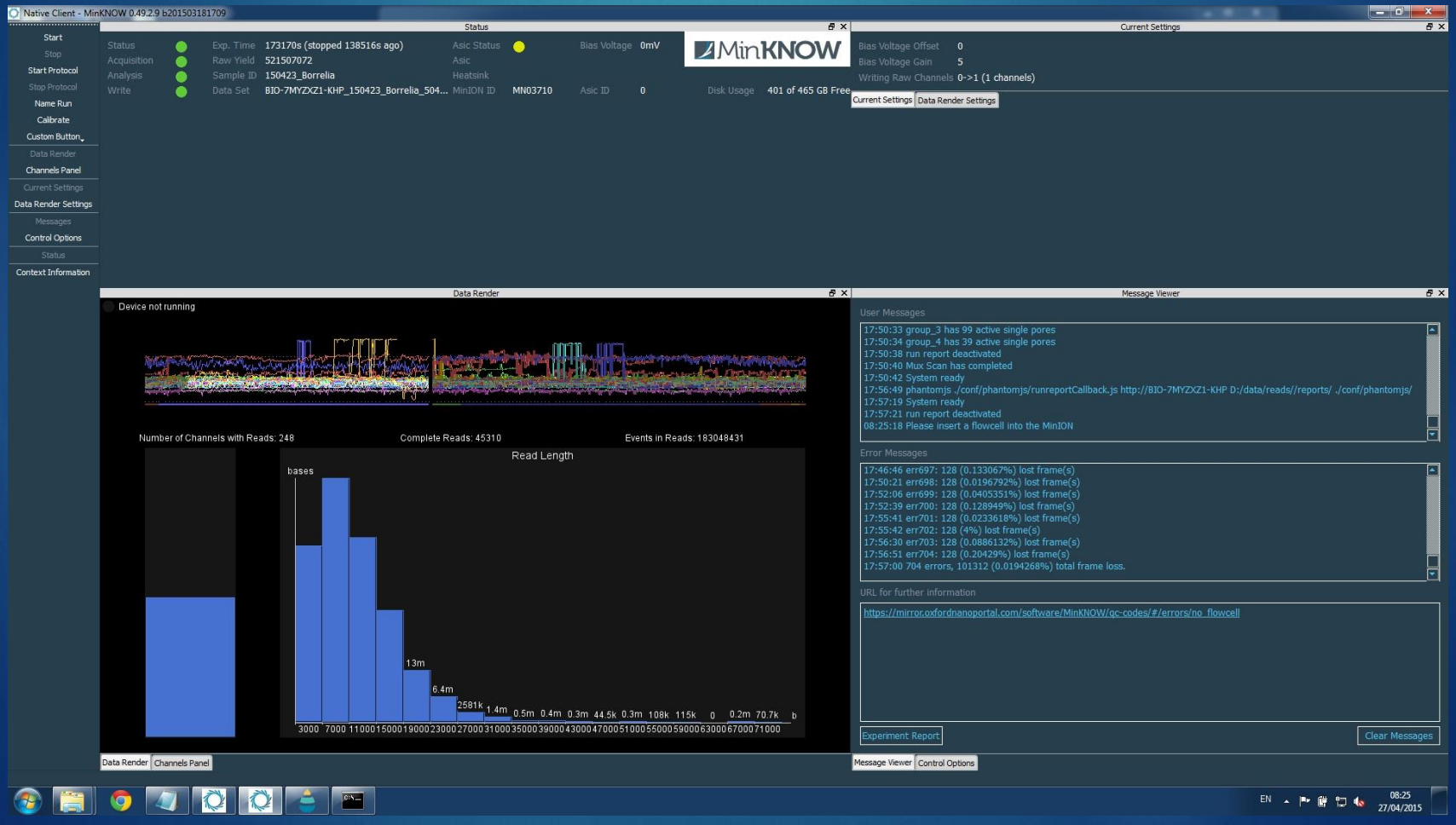
Nanopore reads



MinKNOW
Monitoring the run in real time
Channels Panel



MinKNOW
Monitoring the run in real time
Data render



Real time streaming of data

Metricore

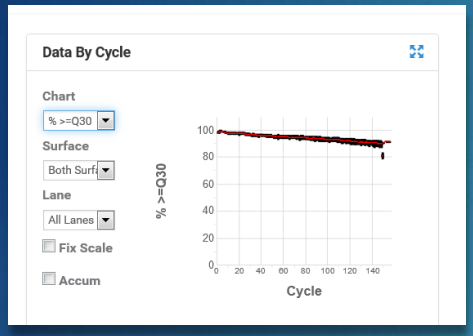
METRICHOR
An Oxford Nanopore Company

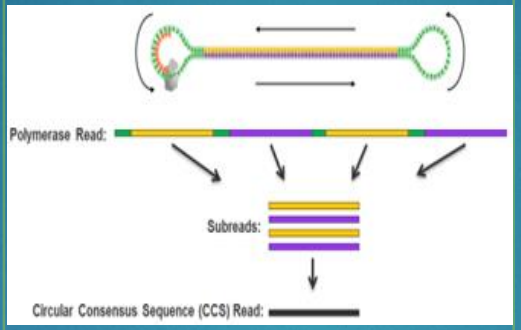
- Developed by ONT
- Converts the voltage profiles "squiggles" into sequence reads
- provides real time streaming of experimental data
- Allows simultaneous analysis of reads until sufficient data is obtained

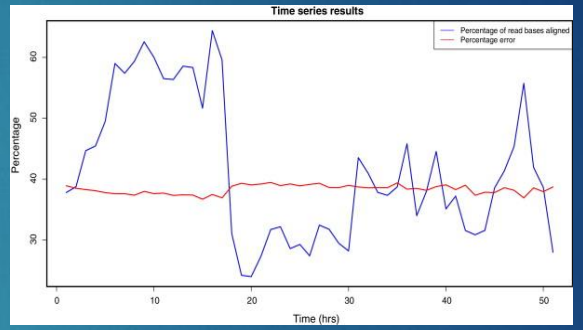
MinoTour

- Developed by Matt Loose at the university of Nottingham

Accuracy of base calling over time







Illumina

The % reads with error rate equal to Q30 (probability of 1:1000 error rate) decreases as sequencing progresses

PacBio

Subreads that are read multiple times in small DNA molecules show no increase in error rate.

MinIon

Plot showing percentage of read bases aligned per hour during the MinION run based on the alignment by LAST and their error rate.

Advantages for research

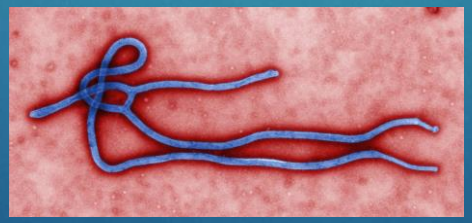
Long-read capability simplifies complete *de novo* assembly of whole genomes

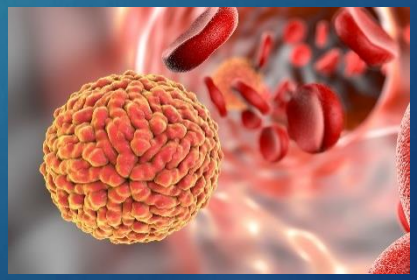
Assessing the performance of the Oxford Nanopore Technologies MinION (2015) T. Laver *et al.*

- allow better genome assembly at lower coverage
- span across repeat regions to improve resolution
- can be used to scaffold short read data assemblies
- potential to resolve large haplotype blocks

Advantages for diagnostics

- Rapid metagenomic identification of viral or bacterial pathogens in clinical samples
- speed up pathogen identification and surveillance monitoring world wide
- already been used to identify a hospital outbreak of *Salmonella* and used for surveillance of Ebola in West Africa, Dengue fever in Indonesia and Zika in Brazil





Disadvantages

- Dependent on a reliable internet connection
- Number of active pores per flowcell is variable

  R6 between 78-484 originally, R7 generally >600 active pores, R9
- Difficulties with high GC genomes
- Version R9 flowcells have higher yield and greater accuracy

  (released end May 2016)
- Error rates are high

  60 x coverage of *E. coli* genome required for a consensus of 99% accuracy using 2D reads

  (MinION Analysis and Reference Consortium: Phase 1 data release and analysis. doi: 10.12688/f1000research.7201.1)

Error rate

Important for Single Nucleotide Polymorphism (SNP) detection

- Percentage of bases not correctly identified when compared to published sequence
- Systematic or random
- Raw reads vs assembled reads

technology	Illumina	PacBio RSII	MinIon	MinIon Mk1
Individual read	0.5% - 100PE 2-5.0% >250 PE	15-20% P6C4	R6 38.2 % [1]	R7 13 % from 2D reads R9 10 % from 2D reads
Assembled reads	Differences to published references genomes.[1] **Species** **SNPs** **Indels** *S. avermitilis* 722 148 *E. coli* 402 17 *B. burgdorferi* 144 16	>97 %	99%[2] using 2D reads x 60 coverage	

1. MinION Analysis and Reference Consortium: Phase 1 data release and analysis. doi: 10.12688/f1000research.7201.1
2. Jain M *et al.* Nature Methods12, 351–356(2015) doi:10.1038/nmeth.3290

Why am I so excited about this?

- Fully portable, very fast and low initial outlay
- Direct RNA sequencing available shortly
- How soon will we be able to sequence DNA:RNA hybrids?
- Many diagnosis could be matter of hours rather than days
- Impact on pathogen detection and surveillance
- CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) monitoring improved
- Impact on education, (universities, collages schools and individuals)
- Space station
- Impact of individual identification

Thank you

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Supported by wellcome trust

http://sequencing.exeter.ac.uk/

Exeter Sequencing Service: MinIon technology

Karen Moore, Audrey Farbos, Jeremie Poschmann, Paul O'Neill and Konrad Paskeiwicz

Oxford Nanopore Technologies have released their MinIon technology to the DNA Sequencing community for beta testing before market release. Exeter Sequencing Service is part of the early access programme.

The MinIon sequencing device offers a portable real time method of sequencing DNA; it is run from a labtop connected to cloud computing. A disposable microfluidic flowcell sits above the device and is controlled directly from below. The prepared DNA library is introduced through a small hole into the flowcell by pipetting.

The flowcell surface is coated with nanopores which sit in an electrically resistant polymer layer. A voltage across the polymer layer results in electric current that moves molecules relative to the current so they pass through the nanopores. As DNA molecules pass through the pore the current is altered and that alteration is dependent on five bases of DNA sequence with in the pore. The current changes are measured in real time and the profile is converted into sequence information via MinIon software and cloud computing. Each nanopore is controlled and measured individually.

The DNA library is prepared directly from genomic DNA without any requirement for amplification. Each double stranded DNA molecule has an adapter attached to one end and a hairpin attached to the other allowing both of the complementary strands to be sequenced as one molecule; this is called a 2D read. Both single reads, complementary reads and 2D reads are generated on the MinIon; 2D reads improve confidence in the data quality as each half or the read should be a compliment of the other. Individual sequence reads may be many kilobases in length. Additional software allows the data to be processed during the run such that reads may be aligned to genomes immediately after the sequence has been processed. This offers a considerable advantage over sequencing methods in terms of speed to results for identification of sequences, strains or species.