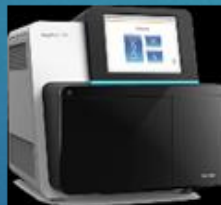


Exeter
sequencing
service

AUDREY FARBOS
JEREMIE POSCHMANN
PAUL O'NEILL
KONRAD PASZKIEWICZ
KAREN MOORE

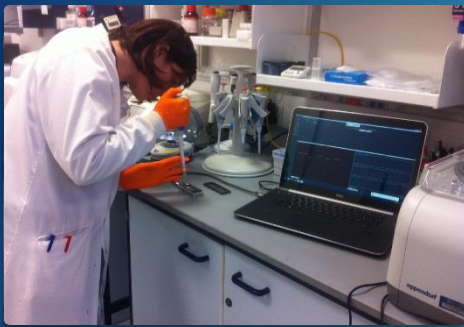
We provide:

- ❖ 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 Minlon Sequencing

Exeter
sequencing
service



Laptop £800

Disable automatic software updates and sleep modes

Software-MinKNOW, Metrichor, MinoTour, Chronolapse

Available storage space >150 Gb

Minlon £1000

- The Minlon 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

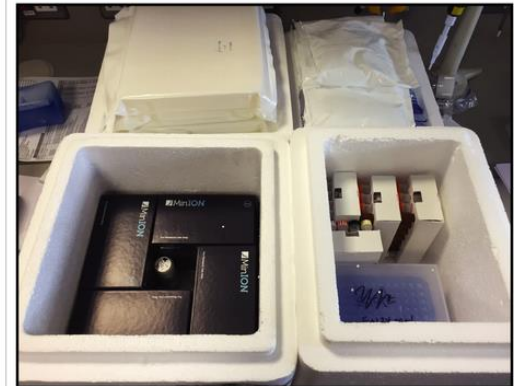


B. Consumables

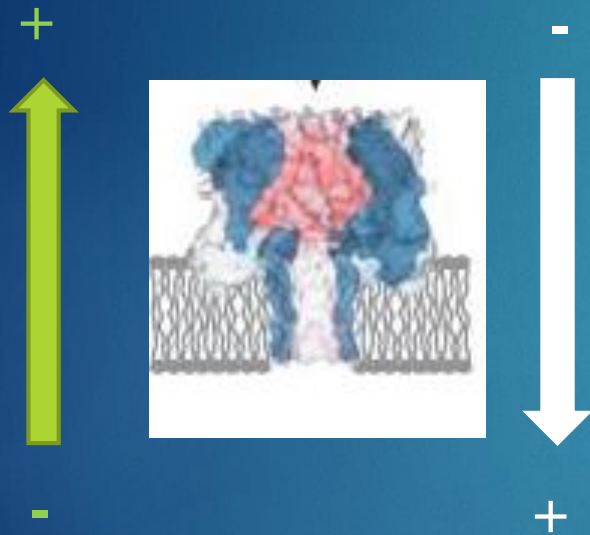
Item	Supplier
DNA LoBind Tubes (2 ml)	Eppendorf
Protein LoBind Tubes (2 ml)	Eppendorf
Qubit Assay Tubes	Life Technologies
PCR Tubes with Flat Caps (0.2 ml)	Starlab
Pipette Tips (10 µl, 20 µl, 100 µl, 200 µl, 1000 µl)	Sarstedt
Nitrile Gloves	Kimberly Clark Professional

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

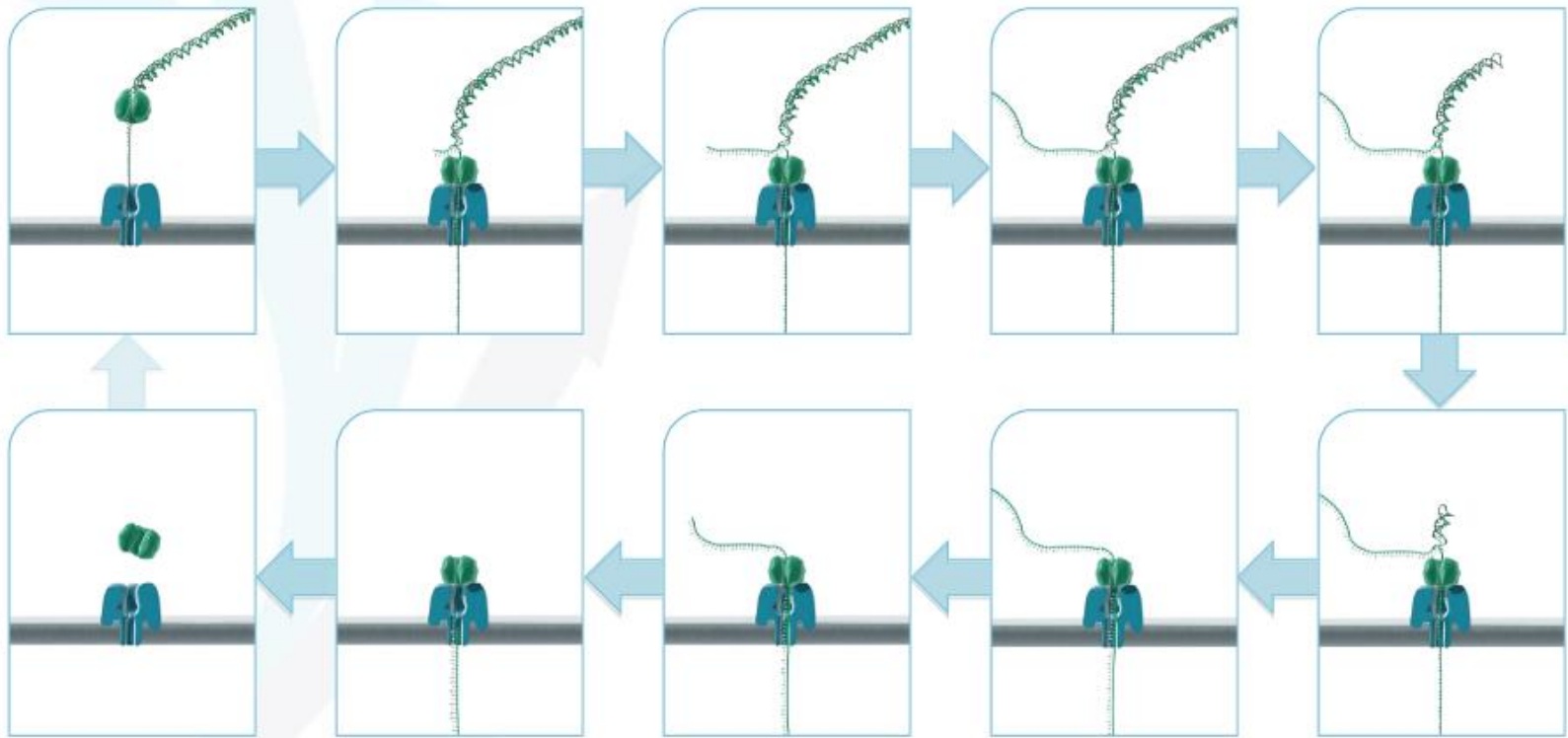


The heart of the matter: Nanopores



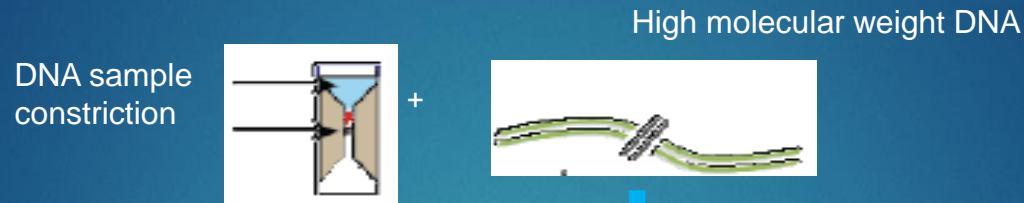
- Nanopores are nanoscale pores embedded in an electrically resistant polymer membrane
- An Application Specific Integrated Circuit controls and measures individual channels corresponding to single nanopores
- A voltage across the membrane sets up an ionic current within the nanopores

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

Library preparation



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



1D template



1D complement



2D - normal



2D - full



MinKNOW

Monitoring the run in real time

Channels Panel

Native Client - MinKNOW 0.49.3.7 b201504271121 - Running ./python/recipes/MAP_4x8hrs_Incrementing_Sequencing_Run.py

Status

Exp. Time 3611s
 Raw Yield 10850752
 Sample ID Chip54_GAERS10_Snutch
 Data Set SNUTCH-GB-BX17_Chip54_GAERS10_Snut...
 MinION ID MN02521

Asic Status ● Bias Voltage -140mV
 Asic 24.46°C
 Heatsink 34.88°C
 Asic ID 3976896244 Disk Usage 95 of 232 GB Free

Current Settings

Bias Voltage Offset 0
 Bias Voltage Gain 5
 Writing Raw Channels 0->1 (1 channels)

Channels Panel

■ saturated (7)
■ -inf to 20pA (0)
■ zero (36)
■ 5pA to +inf (119)
■ Single Pore (10)
■ strand (292)
■ unavailable (46)
■ multiple (2)

125	121	117	113	109	105	101	97	93	89	85	81	77	73	69	65	61	57	53	49	45	41	37	33	29	25	21	17	13	9	5	1
126	122	118	114	110	106	102	98	94	90	86	82	78	74	70	66	62	58	54	50	46	42	38	34	30	26	22	18	14	10	6	2
127	123	119	115	111	107	103	99	95	91	87	83	79	75	71	67	63	59	55	51	47	43	39	35	31	27	23	19	15	11	7	3
128	124	120	116	112	108	104	100	96	92	88	84	80	76	72	68	64	60	56	52	48	44	40	36	32	28	24	20	16	12	8	4
253	249	245	241	237	233	229	225	221	217	213	209	205	201	197	193	189	185	181	177	173	169	165	161	157	153	149	145	141	137	133	129
254	250	246	242	238	234	230	226	222	218	214	210	206	202	198	194	190	186	182	178	174	170	166	162	158	154	150	146	142	138	134	130
255	251	247	243	239	235	231	227	223	219	215	211	207	203	199	195	191	187	183	179	175	171	167	163	159	155	151	147	143	139	135	131
256	252	248	244	240	236	232	228	224	220	216	212	208	204	200	196	192	188	184	180	176	172	168	164	160	156	152	148	144	140	136	132
381	377	373	369	365	361	357	353	349	345	341	337	333	329	325	321	317	313	309	305	301	297	293	289	285	281	277	273	269	265	261	257
382	378	374	370	366	362	358	354	350	346	342	338	334	330	326	322	318	314	310	306	302	298	294	290	286	282	278	274	270	266	262	258
383	379	375	371	367	363	359	355	351	347	343	339	335	331	327	323	319	315	311	307	303	299	295	291	287	283	279	275	271	267	263	259
384	380	376	372	368	364	360	356	352	348	344	340	336	332	328	324	320	316	312	308	304	300	296	292	288	284	280	276	272	268	264	260
509	505	501	497	493	489	485	481	477	473	469	465	461	457	453	449	445	441	437	433	429	425	421	417	413	409	405	401	397	393	389	385
510	506	502	498	494	490	486	482	478	474	470	466	462	458	454	450	446	442	438	434	430	426	422	418	414	410	406	402	398	394	390	386
511	507	503	499	495	491	487	483	479	475	471	467	463	459	455	451	447	443	439	435	431	427	423	419	415	411	407	403	399	395	391	387
512	508	504	500	496	492	488	484	480	476	472	468	464	460	456	452	448	444	440	436	432	428	424	420	416	412	408	404	400	396	392	388

Show Channels by State Freeze OFF OFF

Show All Channels Hide All Channels Select Surrounding 8 Channels Mode OFF

User Messages

follows
 08:34:04 group_1 has 481 active single pores
 08:34:05 group_2 has 405 active single pores
 08:34:06 group_3 has 263 active single pores
 08:34:07 group_4 has 87 active single pores
 08:34:28 run report deactivated
 08:34:29 Mux Scan has completed
 08:34:32 System ready
 08:34:39 Valid flowcell detected.

Error Messages

URL for further information

<https://wiki.nanoporetech.com/display/HOME/MAP+Community/>

Experiment Report Clear Messages

2015-04-30 08:34:57.10

MinKNOW

Monitoring the run in real time

Data render

The screenshot displays the MinKNOW software interface, which is used for monitoring and controlling a MinION sequencing device. The interface is divided into several panels:

- Status Panel:** Shows the current state of the device. The status is "Device not running". Key metrics include: Exp. Time (173170s, stopped 138516s ago), Raw Yield (521507072), Sample ID (150423_Borrella), Data Set (BIO-7MYZKZ1-KHP_150423_Borrella_504...), MinION ID (MN03710), Asic ID (0), Bias Voltage (0mV), and Disk Usage (401 of 465 GB Free).
- Data Render Panel:** Displays a real-time visualization of sequencing data. It shows a "Number of Channels with Reads: 248", "Complete Reads: 45310", and "Events in Reads: 183048431". A histogram shows the distribution of read lengths, with the x-axis labeled "bases" and the y-axis labeled "b". The histogram shows a peak around 7000 bases, with a smaller peak at 13m (13,000,000) and another at 6.4m (6,400,000). The x-axis ranges from 3000 to 71,000 bases.
- User Messages Panel:** Displays a log of system messages, including: "17:50:33 group_3 has 99 active single pores", "17:50:34 group_4 has 39 active single pores", "17:50:38 run report deactivated", "17:50:40 Mux Scan has completed", "17:50:42 System ready", "17:56:49 phantomjs ./conf/phantomjs/runreportCallback.js http://BIO-7MYZKZ1-KHP_D:/data/reads/reports/.conf/phantomjs/", "17:57:19 System ready", "17:57:21 run report deactivated", and "08:25:18 Please insert a flowcell into the MinION".
- Error Messages Panel:** Displays a log of error messages, including: "17:46:46 err697: 128 (0.133067%) lost frame(s)", "17:50:21 err698: 128 (0.0196792%) lost frame(s)", "17:52:06 err699: 128 (0.0405351%) lost frame(s)", "17:52:39 err700: 128 (0.128949%) lost frame(s)", "17:55:41 err701: 128 (0.0233618%) lost frame(s)", "17:55:42 err702: 128 (4%) lost frame(s)", "17:56:30 err703: 128 (0.0886132%) lost frame(s)", "17:56:51 err704: 128 (0.20429%) lost frame(s)", and "17:57:00 704 errors, 101312 (0.0194268%) total frame loss".
- URL for further information:** Provides a link to the MinKNOW error codes page: https://mirror.oxfordnanoportal.com/software/MinKNOW/qc-codes/#/errors/no_flowcell.

The interface also includes a navigation menu on the left with options like Start, Stop, Start Protocol, Stop Protocol, Name Run, Calibrate, Custom Button, Data Render, Channels Panel, Current Settings, Data Render Settings, Messages, Control Options, Status, and Context Information. The bottom of the screen shows the Windows taskbar with various application icons and the system clock indicating 08:25 on 27/04/2015.

Real time streaming of data

Metricore



- ▶ 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

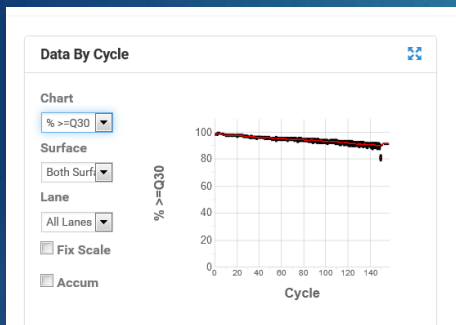
MinoTour



- ▶ Developed by Matt Loose at the university of Nottingham

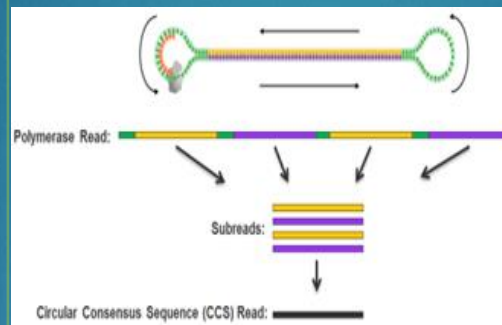


Accuracy of base calling over time



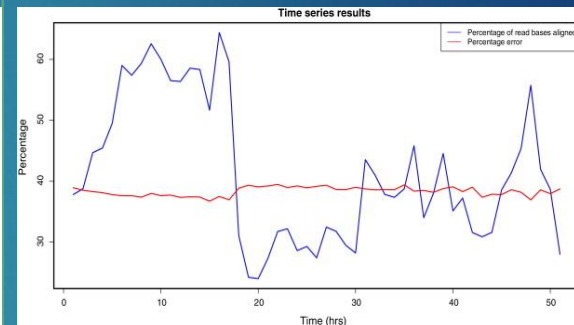
Illumina

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.

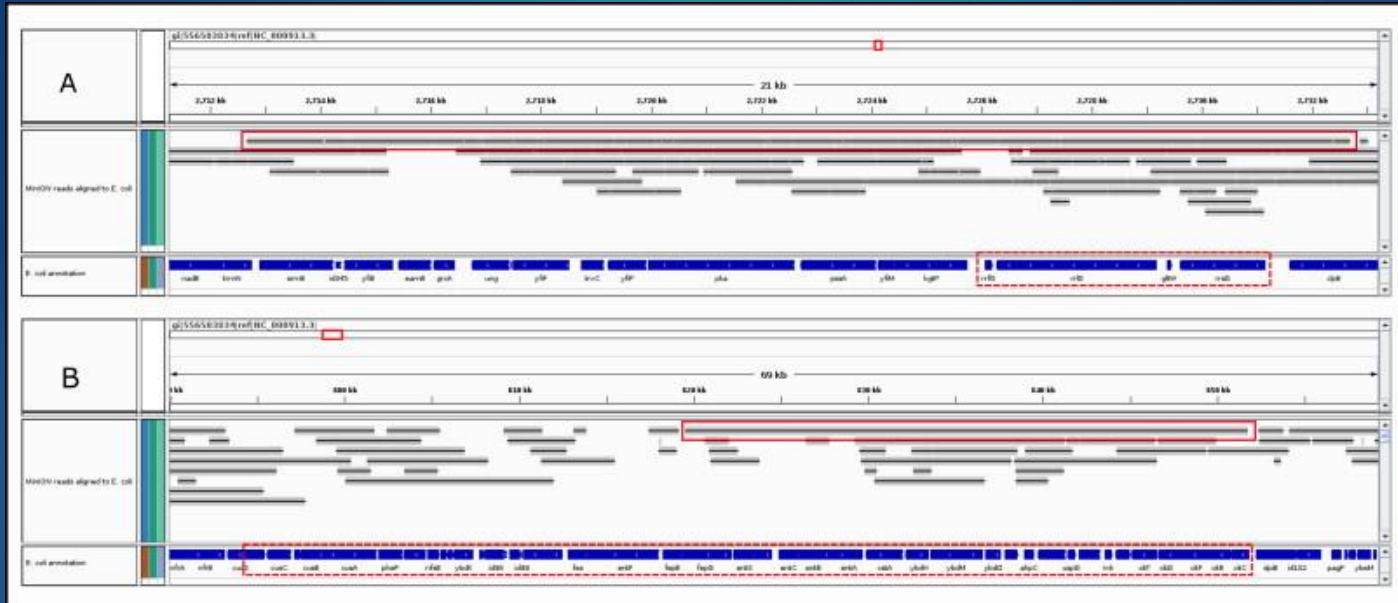


Minlon

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

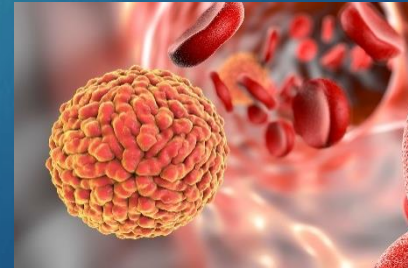
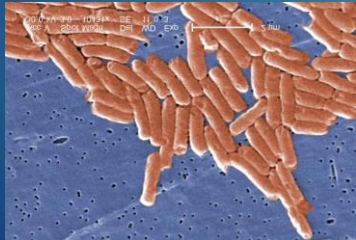


Assessing the performance of the Oxford Nanopore Technologies MinION (2015) T. Laver *et al.*

- 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	Minlon	Minlon 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 <table border="1"><thead><tr><th>Species</th><th>SNPs</th><th>Indels</th></tr></thead><tbody><tr><td><i>S. avermitilis</i></td><td>722</td><td>148</td></tr><tr><td><i>E. coli</i></td><td>402</td><td>17</td></tr><tr><td><i>B. burgdorferi</i></td><td>144</td><td>16</td></tr></tbody></table>	Species	SNPs	Indels	<i>S. avermitilis</i>	722	148	<i>E. coli</i>	402	17	<i>B. burgdorferi</i>	144	16	>97 %	99% ² using 2D reads x 60 coverage	
Species	SNPs	Indels														
<i>S. avermitilis</i>	722	148														
<i>E. coli</i>	402	17														
<i>B. burgdorferi</i>	144	16														

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, colleges schools and individuals)
- ▶ Space station
- ▶ Impact of individual identification

Thank you

AUDREY FARBOS

JEREMIE POSCHMANN

PAUL O'NEILL

KONRAD PASZKIEWICZ

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

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UNIVERSITY OF
EXETER

Exeter
sequencing
service

MRC

Cloud Infrastructure
for Microbial
Bioinformatics



Oxford

NANOPORETM

Technologies



Exeter Sequencing Service: Minlon technology

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



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

The Minlon sequencing device offers a portable real time method of sequencing DNA; it is run from a laptop 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 within the pore. The current changes are measured in real time and the profile is converted into sequence information via Minlon 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 Minlon; 2D reads improve confidence in the data quality as each half of the read should be a complement 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.