Advances in Nanopore Technology
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IFSH HTS Symposium 19th May 2022
Agenda

• Company overview
• The heart of our technology
• Why nanopore sequencing?
• Conclusion
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Life Science Research

Our company vision and global impact
Nanopore sequencing technology is well established globally
Celebrating the work of the Nanopore Community

...who have used nanopore technology in over

2,355

publications to date

From the very small...

28 kb

virus genome

...to the largest assembled animal genome...

43 Gb

lungfish genome

...to the complete sequence of a human genome...

3.055 Gb

The number of publications has grown and grown...

Cumulative number of publications


And as throughput has grown, so has the range of applications:

No. of publications by grouping (to end 2016)⁶

- 62% Microbiology
- 4% Environmental
- 1% Animal
- 3% Plant
- 21% Bioinformatics
- 4% Human (other translational)
- 4% Human (cancer)
- 1% Human genetics

No. of publications by grouping (to date)⁵

- 47% Microbiology
- 6% Environmental
- 12% Animal
- 9% Plant
- 12% Bioinformatics
- 7% Human (other translational)
- 3% Human (cancer)
- 4% Human genetics

Read the latest publications at

nanoporetech.com/resource-centre

1. As of 14th November 2021; includes pre-prints and reviews
4. Mark et al. bioRxiv; DOI: http://dx.doi.org/10.1101/2021.05.28.487086 (2021)
5. Over 7 days; 9th-12th March 2021
6. Some publications may be tagged in more than one category
The heart of our technology
The heart of our technology

The nanopore

- In nature, protein nanopores function as gateways between two systems
- We have carefully engineered protein nanopores through mutating key residues in the barrel of the pore
Nanopore DNA/RNA sequencing
A DNA/RNA strand is passed through a nanopore: an electrical signal is interpreted into sequence data.

- DNA passes through nanopore at 400+ bases per second
- Enzyme fuel and co-factors
- Run conditions (salt, pH, temperature)
- Nanopores, allowing ion flow, are created in a membrane
- Passage of DNA and RNA bases (including modified bases) through the pore creates characteristic disruptions in the current
- Data is instantly generated and analysable
Why nanopore sequencing?
Why nanopore sequencing?
Access a wide range of unique benefits

Direct sequencing of DNA or RNA
- Native PCR-free sequencing: processes read lengths presented rather than generating read lengths
- No light-based sequencing steps or surrogate markers required
- Base modification e.g. methylation information is also collected

Read short to ultra-long sequences
- Widest application range possible
- Long reads enable quick and easy elucidation of genomic variations: SVs; repeat regions; phasing; and transcript isoform resolution
- Longest read length: >4 Mb

REAL real-time analysis!
- For each flow cell, start and stop as required; no fixed run time
- Additional flow cells can be added whilst a run is in progress (no batching required)
- Adaptive sampling: on-sequencer target enrichment; reject or accept reads on a strand by strand basis

[Note: Access to raw data enables reanalysis of legacy data; future proofing purchase]
Method: Adaptive sampling
On-flow-cell enrichment, with no limit to read length or target number

Strand approaches nanopore

Region of interest
- Strand is sequenced and analysed in real time
- Region of interest found. Sequencing continues
- Nanopore completes sequencing, available for next strand

No region of interest
- Strand is sequenced and analysed in real time
- Sequence seen is not in region of interest. Strand ejected. Nanopore available for next strand
- Subsequent strand sequenced (shown here with region of interest)
Scalable: portable to desktop

Easy transition from small scale to large scale
MinION Mk1B
Portable, highly accessible DNA/RNA sequencing
MinION Mk1C
Portable, fully integrated DNA/RNA sequencing
Flongle
Cost-effective, single-use flow cells
GridION Mk1

A self-contained benchtop sequencer with on-board powerful compute
A broad range of devices: one core technology
Invest in the technology, not just the box
A broad range of devices: one core technology
Invest in the technology, not just the box

MinION
Mk1B
Portable, highly accessible DNA/RNA sequencing
TMO: Up to 50 Gb*

MinION
Mk1C
Portable, fully integrated DNA/RNA sequencing
TMO: Up to 50 Gb*

GridION
Mk1
Self-contained bench-top sequencer with powerful compute
TMO: Up to 250 Gb*

PromethION
P24
P48
The most powerful benchtop sequencer (P24 or P48)
TMO: Up to 290 Gb per flow cell*

Flongle
Adapter for MinION/GridION, supports smaller single-use flow cells.
TMO: Up to 2.8 Gb

* TMO: Theoretical Max. Output when system is run for 72 hours (or 16 hours for Flongle) at 420 bases / second. Outputs may vary according to library type, run conditions etc.

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Conclusion
Nanopore-only bacterial genomes from pure culture

Q20+ chemistry

Oxford Nanopore R10.4 long-read sequencing enables near-perfect bacterial genomes from pure cultures and metagenomes without short-read or reference polishing

Mantas Sereika, Rasmus Hansen Kirkegaard, Seren Michael Karst, Thomas Yssing Michaelsen, Emil Aarre Sorensen, Rasmus Dam Wollenberg, Mads Albertsen

do: https://doi.org/10.1101/2021.10.27.466057

This article is a preprint and has not been certified by peer review (what does this mean?).

Using the new Q20+ chemistry and R10.4 flow cells, Sereika et al. have been able to generate near-perfect bacterial genomes.

- 99% modal raw read accuracy
- More accurate consensus calling of homopolymers
- Polishing with short reads yielded almost no improvement in genome quality

https://www.biorxiv.org/content/10.1101/2021.10.27.466057v2
<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
</tr>
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<tbody>
<tr>
<td>Raw-read accuracy</td>
<td>99.6 %, Q23&lt;sup&gt;x&lt;/sup&gt;</td>
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<tr>
<td>Duplex</td>
<td>99.92%, Q31&lt;sup&gt;x&lt;/sup&gt;</td>
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<tr>
<td>SNP detection (F1 scores human)</td>
<td>SNV: 99.9 % Indel: 90 %</td>
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<tr>
<td>Assembly (Human)</td>
<td>80 Mbase N50&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Q 47&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Assembly (Bacterial)</td>
<td>Circular&lt;sup&gt;3&lt;/sup&gt; &gt; Q 50</td>
</tr>
<tr>
<td>SV detection (F1 scores human)</td>
<td>96%</td>
</tr>
<tr>
<td>5mC methylation (also available)</td>
<td>99.8 %&lt;sup&gt;z&lt;/sup&gt; (5hmC, 6mA)</td>
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Test accuracy, e.g. LamPORE:
Sensitivity 99.1 %, Specificity 99.6 %

Instrument record (int):
10 Tbase output, 48 flow cells
Flow cell records:
307 Gbases (int)<sup>y</sup>, 245 Gbases (ext)

Read length records:
4.2 Mbases (int), 2.3 Mbases (ext)
Longest Q40 read<sup>x</sup>:
143 kbases
Longest perfect read<sup>x</sup>:
72 kbases
https://nanoporetech.com/resource-centre

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Thank you