DNA sequencing

First generation sequencing

Sequencing many copies of one molecule

Sanger sequencing

Second generation sequencing

Sequencing many copies of many molecules in a **massively** parallel fashion

Illumina

>> Current technology

Third generation sequencing

Sequencing **single** molecules in a (massively?) parallel fashion

Helicos tsms tm – single molecule illumina SMRT sequencing – zero mode waveguide Nanopore (MinION) – nano pores in membrane

Nanopore

Passing single molecules through engineered nano-scale pores in membrane and measuring the changes in ionic current

Real time (pause-able/interruptible)

Long reads (determined by DNA length) Very fast, simple sample prep (no PCR/copying step)

Low initial cost (cost per run?)

DNA can be sequenced by threading it through a microscopic pore in a membrane. Bases are identified by the way they affect ions flowing through the pore from one side of the membrane to the other.



Flow cell



MinION



MinION - DNA





Issues:

Accuracy!

- High error rate
- Better on long sequences?
- Can be overcome through coverage?

Cost

- > Flow cells are replaced (for now) for free
- Sample preparation kits (~\$150/run)

Test Run - 1MBp Plasmid – Running





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Software to interpret data from MinION:

Basecalling software: Poretools:

- Reads native .fast5 files
- Converts to .fasta, .fastq
- Provides statistics Albacore:
- Neural net

Assembly software:

- SPAdes
- SMARTdenovo
- Canu
- >> Software is open source
- >> Not supported
- >> Not user friendly

Test Run - 1MBp Plasmid – Results

Graphic Summary



Graphics -

Shigella sonnei strain SS084469 plasmid pSH4469, complete sequence

GenBank: KJ406378.1

GenBan	k FASTA									
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	tnpR	unuC	ygaA AI	T413321	trbA	tro	U RI	T41373.1	AIT41380.1	pilL
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https://www.ncbi.nlm.nih.gov/nuccore/696158084?report=graph

In the near future:

Bento Lab: A DNA laboratory for everybody by Bento Lab - Kickstarter

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VoITRAX VoITRAX Introduction Programme

About VolTRAX

Oxford Nanopore offers a range of options for converting your original biological sample to a form ready for application into a nanopore sensing device.

Oxford Nanopore has developed VoITRAX a small device designed to perform library preparation automatically, so that a user can get a biological sample ready for analysis, hands free.



About SmidgION



Accessible tech

Nanopore sensing technology can uniquely The end-to-end process of sample be miniaturised for portable analysis of DNA preparation, measurement and analysis for and other biological molecules. The handheld nanopore technology is become increasingly MinION is already established for portable simplified. For example, new kits allow ten DNA sequencing. Oxford Nanopore has now minute library preparation, the VolTRAX will started developing an even smaller device, provide programmable hands-off sample SmidgION. preparation, and Oxford Nanopore provides analysis workflows such as 'What's in my

Mobile analyses

SmidgION uses the same core nanopore sensing technology as MinION and PromethION but will be designed for use with smartphones or other mobile, low power devices. It is designed to cater for a broad range of field-based analyses; pot ential applications may include remote monitoring of pathogens in a breakout or infectious disease; the on-site analysis of environmental samples such as water/metagenomics samples, real time species ID for analysis of food, timber, wildlife or even unknown samples; field-based analysis of agricultur al environments, and much more.

Smidg**ION**

More Nanopore Products

https://www.kickstarter.com/projects/339005690/bento-lab-a-dna-laboratory-for-everybody

Page 2 of 13 https://nanonoretech.com/products/voltrax Page 1 of 4

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SmidgION: nanopore sensing for use with mobile devices

Using the same core technology as the handheld MinION device, we are now starting to develop an even smaller device.

Pot' (WIMP). As end-to-end analysis

people and in more locations.

becomes more accessible, the SmidgION is

being designed to allow analyses by more