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

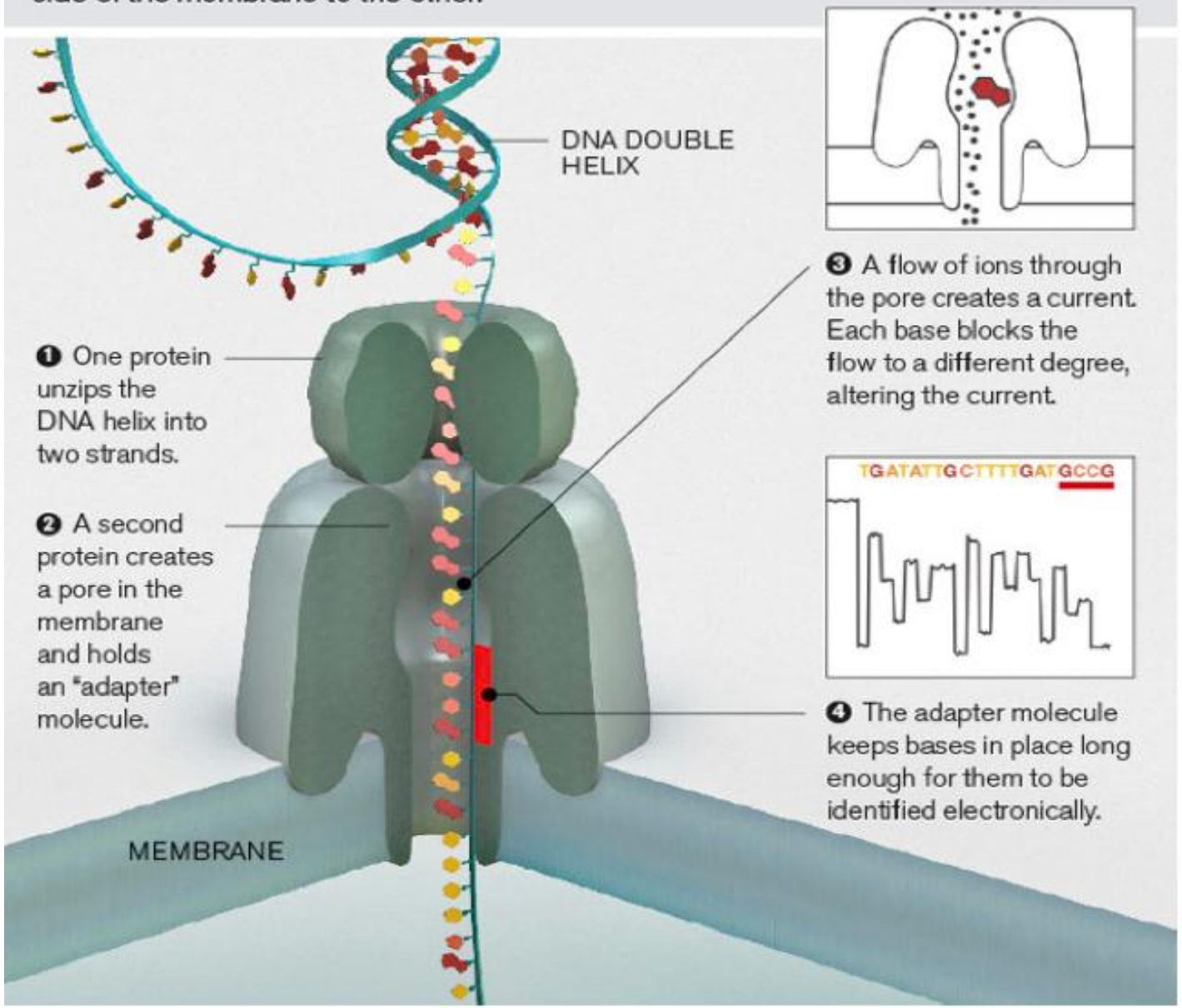
Real time (pause-able/interruptible)

Long reads (determined by DNA length)

Very fast, simple sample prep (no PCR/copying step)

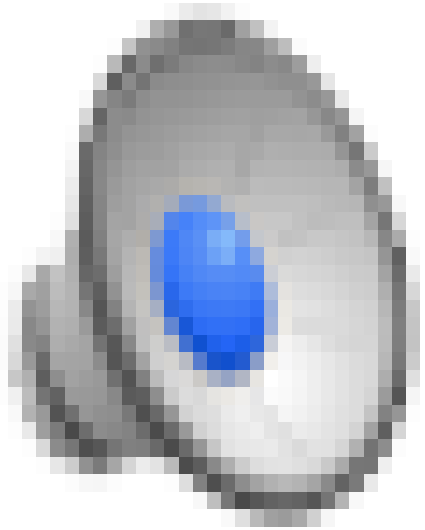
Low initial cost (cost per run?)

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.

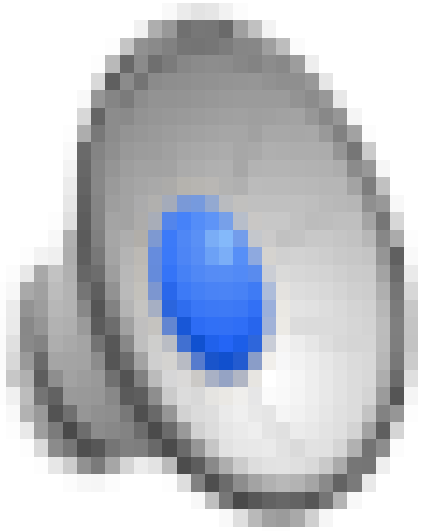


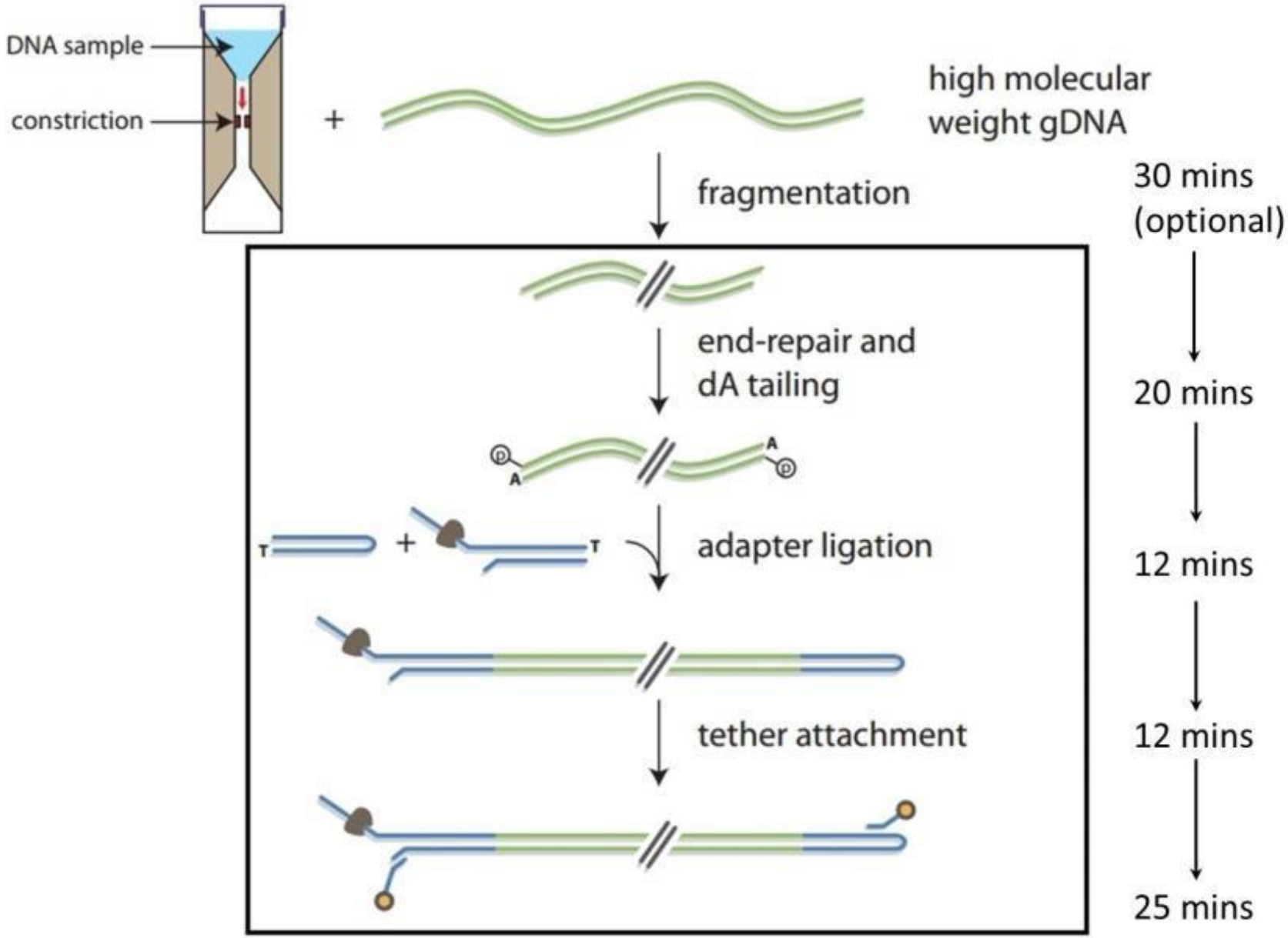
Flow cell

MinION



MinION - DNA




DNA sample
constriction
+
high molecular weight gDNA
fragmentation
30 mins (optional)
end-repair and dA tailing
20 mins
A
adapter ligation
12 mins
tether attachment
12 mins
25 mins

Issues:

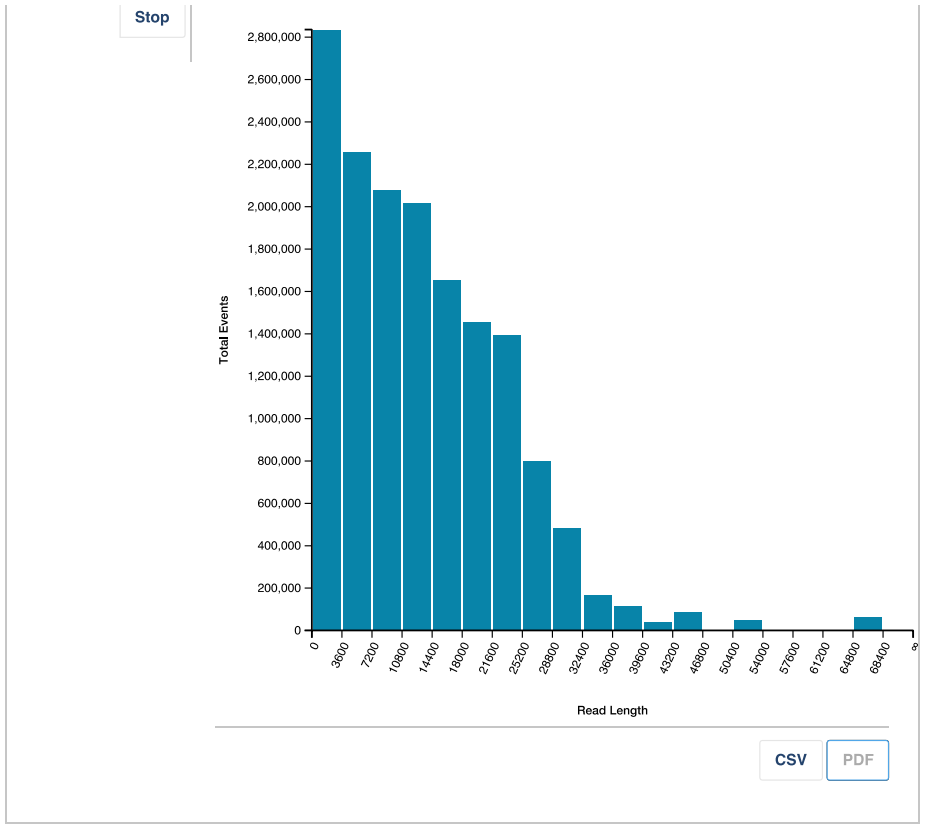
Accuracy!

- 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



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

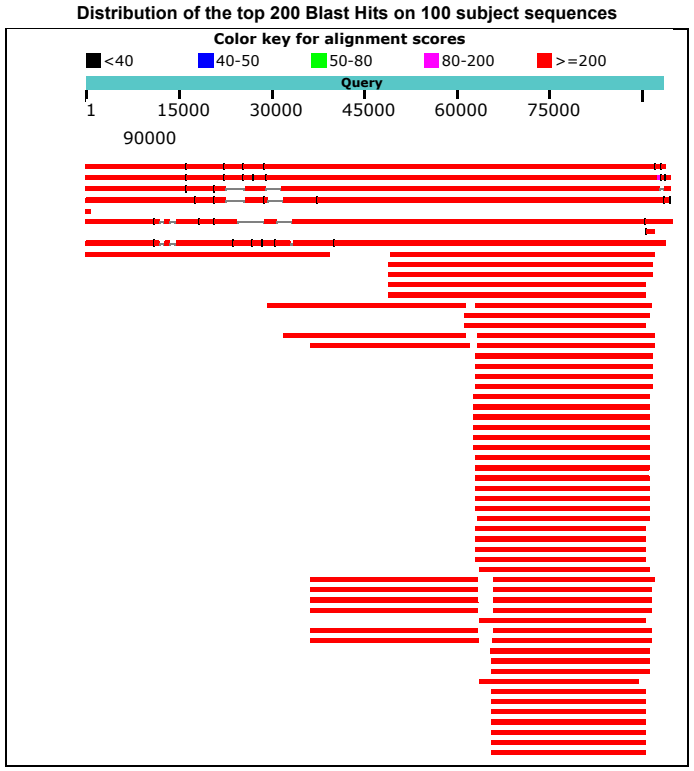
>> Software is open source

>> Not supported

>> Not user friendly

Test Run - 1MBp Plasmid – Results

Graphic Summary

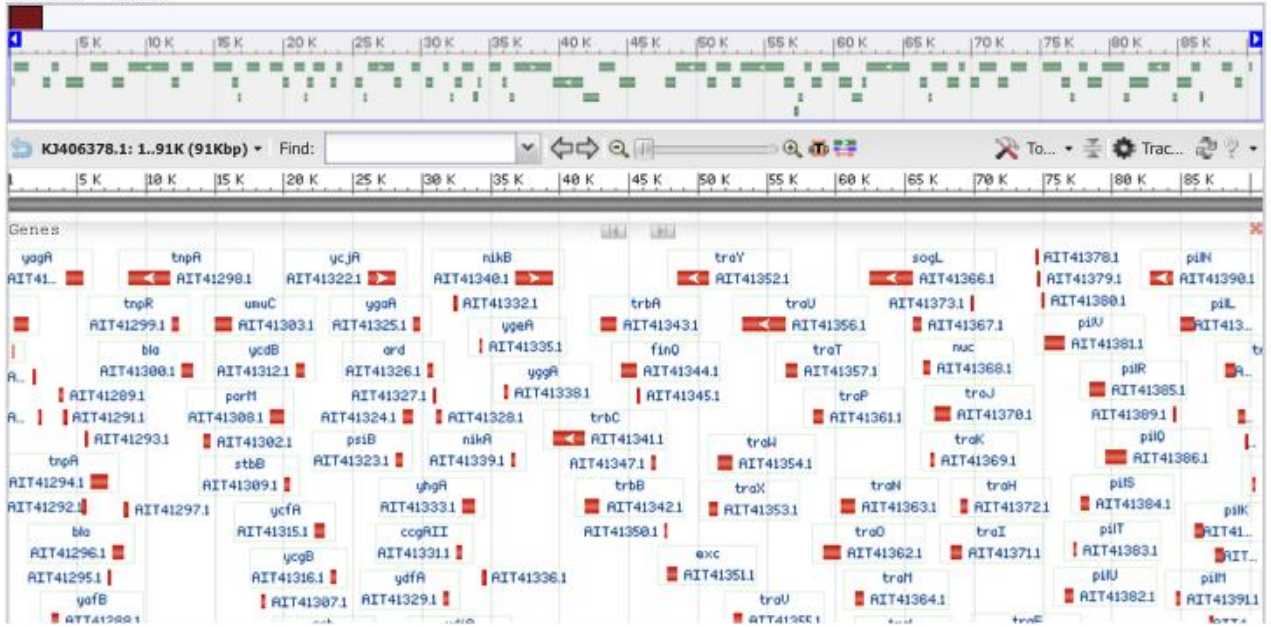


Graphics

Shigella sonnei strain SS084469 plasmid pSH4469, complete sequence

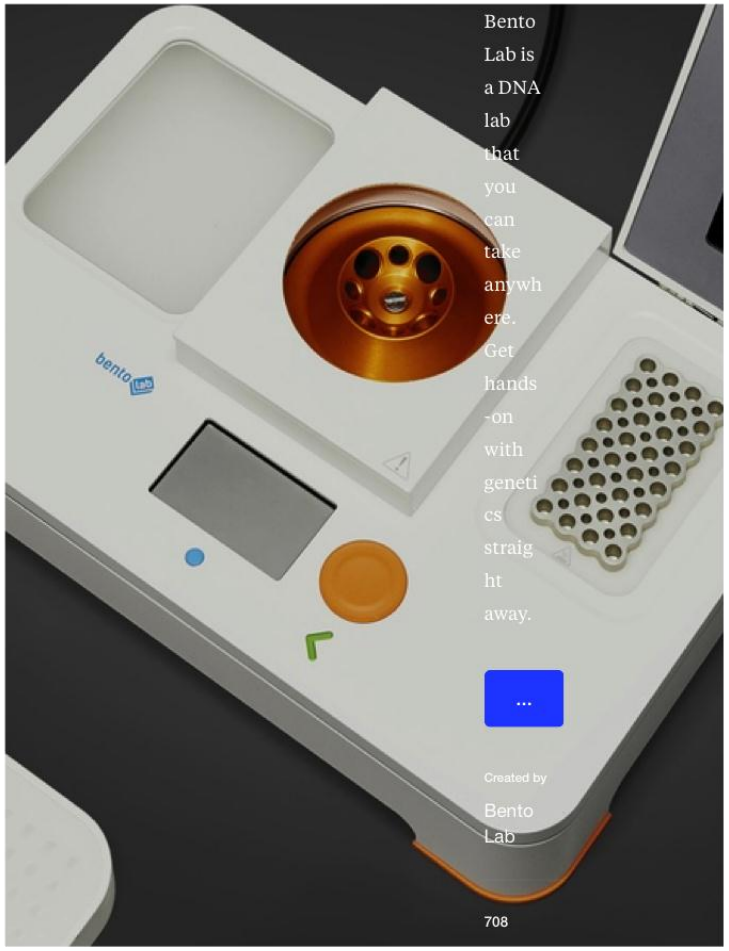
GenBank: KJ406378.1

GenBank FASTA



https://www.ncbi.nlm.nih.gov/nuccore/696158084?report=graph

In the near future:

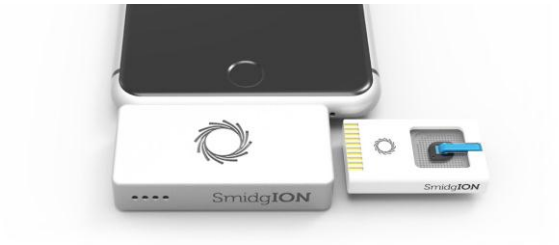


VolTRAX VolTRAX Introduction Programme

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 VolTRAX – a small device designed to perform library preparation automatically, so that a user can get a biological sample ready for analysis, hands free.



SmidgION: nanopore sensing for use with mobile devices

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

SmidgION

About SmidgION

Nanopore sensing technology can uniquely be miniaturised for portable analysis of DNA and other biological molecules. The handheld MinION is already established for portable DNA sequencing. Oxford Nanopore has now started developing an even smaller device, SmidgION.

Accessible tech

The end-to-end process of sample preparation, measurement and analysis for nanopore technology is become increasingly simplified. For example, new kits allow ten minute library preparation, the VolTRAX will provide programmable hands-off sample preparation, and Oxford Nanopore provides analysis workflows such as 'What's in my Pot' (WIMP). As end-to-end analysis becomes more accessible, the SmidgION is being designed to allow analyses by more people and in more locations.

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; potential 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 agricultural environments, and much more.

More Nanopore Products