Oxford Nanopore sequencing

Oxford Nanopore sequencing

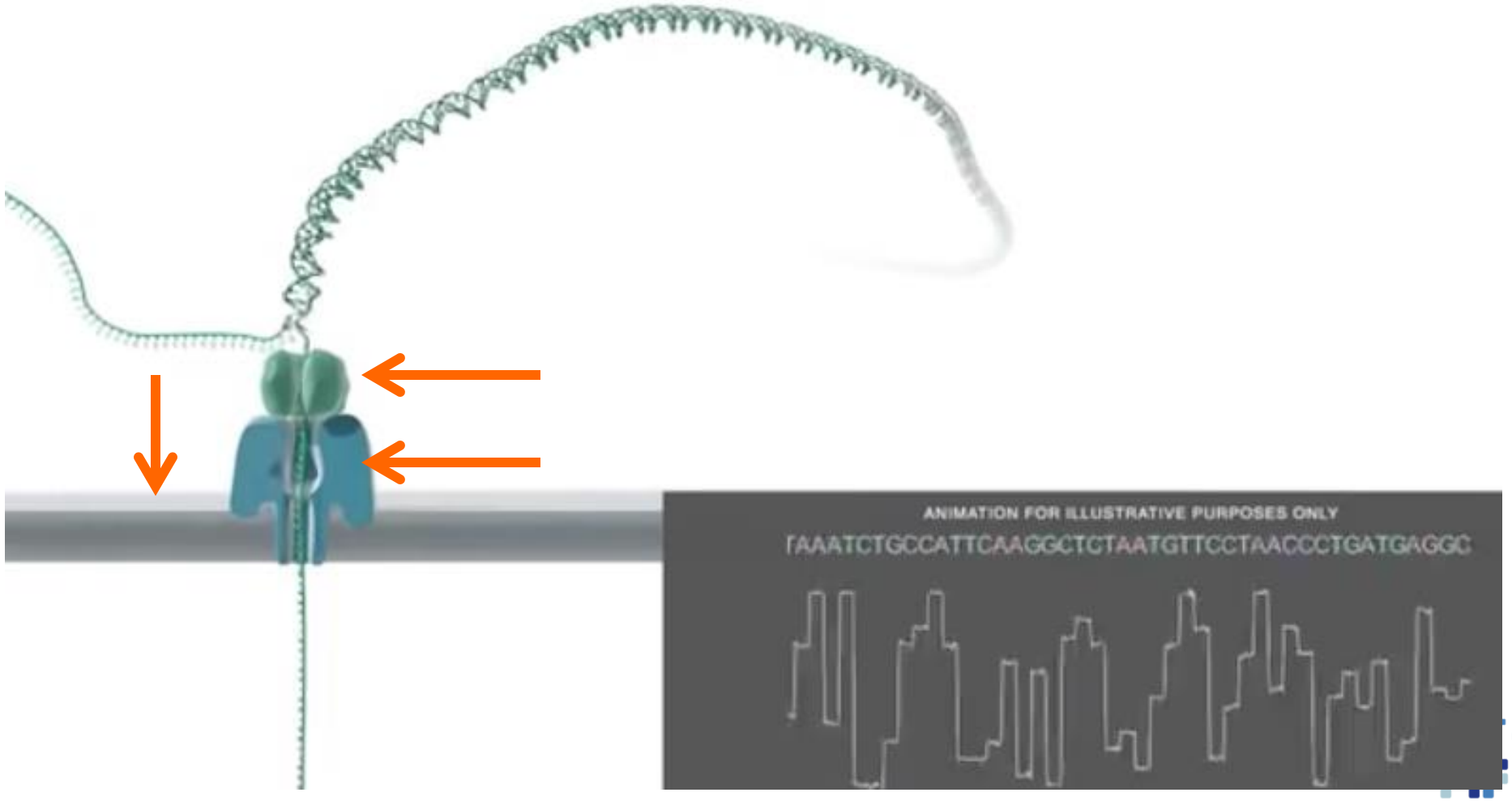


Image c/o Oxford Nanopore, https://www.youtube.com/watch?v=3UHw22hBpAk

Oxford Nanopore sequencing

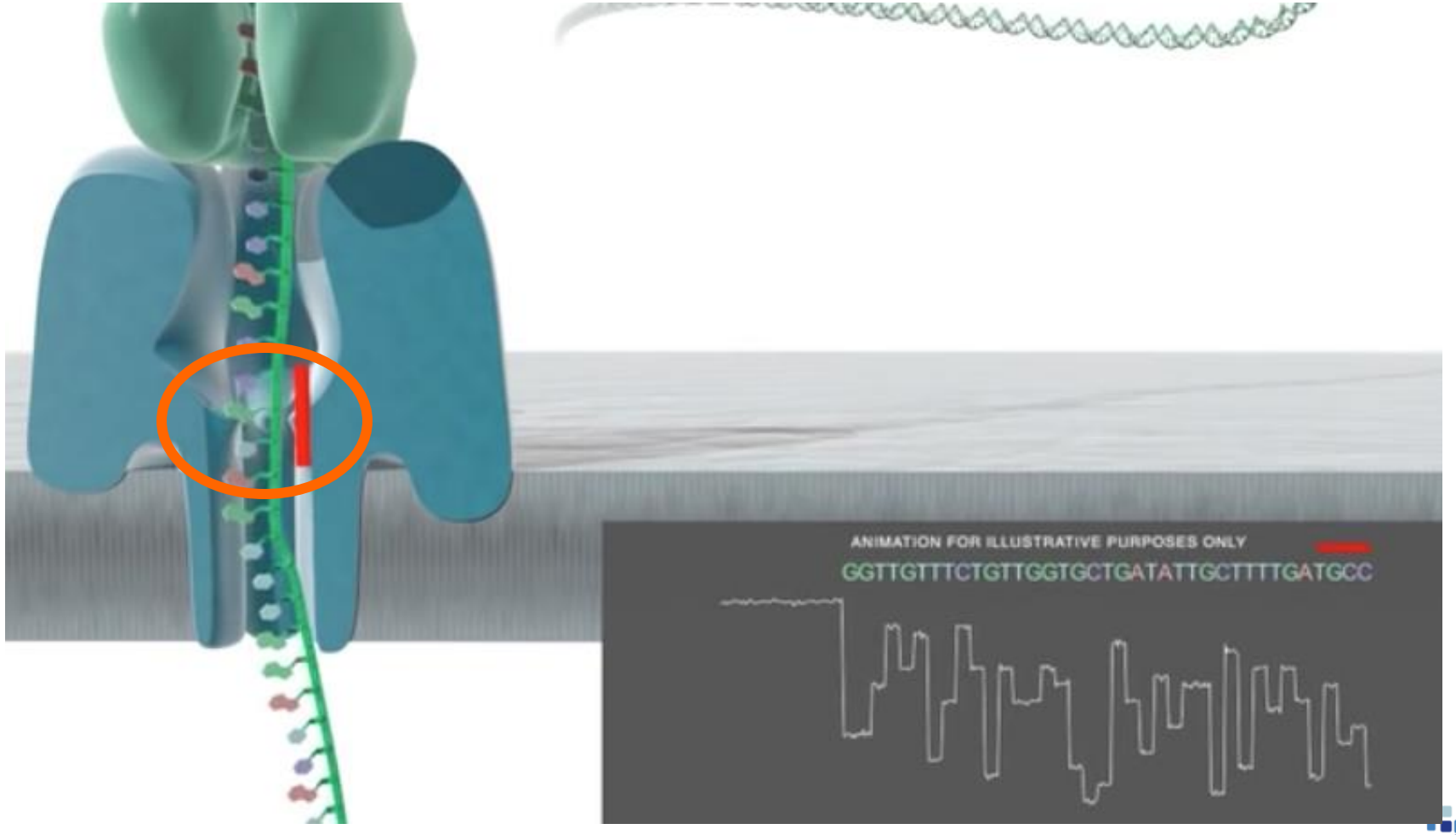


Image c/o Oxford Nanopore, https://www.youtube.com/watch?v=3UHw22hBpAk

Recent accuracy improvements

Image c/o Oxford Nanopore

Q20+

- Combination of latest Nanopore (R10.4.1) and Library Construction kit (“Kit 14”) will give single strand accuracy of “99.3%” and duplex (where both strands of DNA are basecalled) of “~99.9%”
- Duplex = methylation information & high quality basecalls from single strand – no need for consensus (which can obscure rare variants)

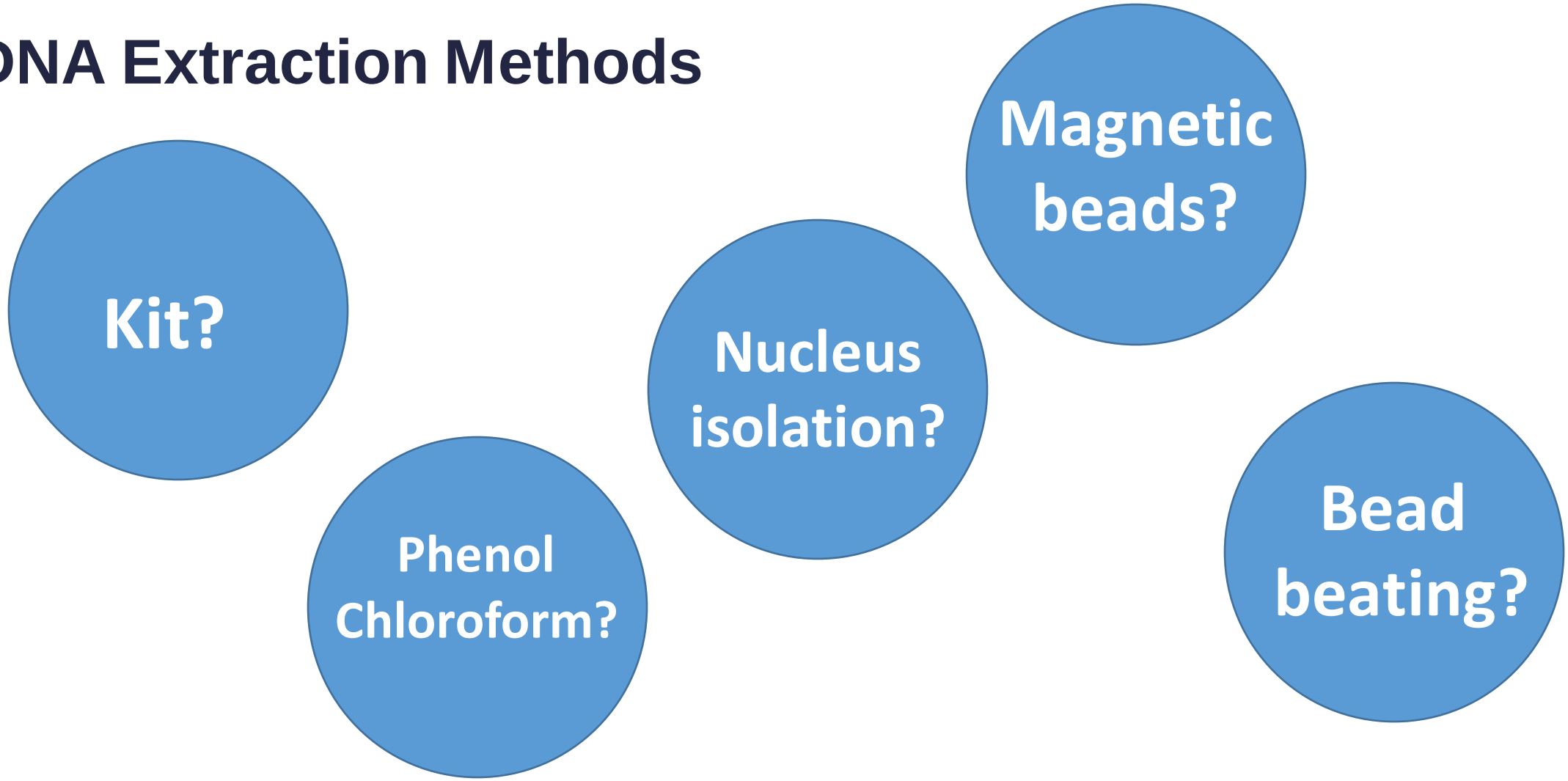
- This is currently in early access testing and not fully commercially available
- Some applications, e.g. Direct RNA sequencing, may not be available as quickly as others

N.B. Illumina NovaSeq stats suggest >75% reads at Q30 at 2x250 or >90% reads at Q30 at 2x50bp

https://emea.illumina.com/systems/sequencing-platforms/novaseq/specifications.html

Q10 = 90% Q20 = 99% Q30 = 99.9%

DNA Extraction Methods



You cannot get long reads from short fragments!

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DNA extraction methods

The four S's!

Safety

Species

Suitable (cost, equipment)

Scale up (or size)

What is the best method for your application?

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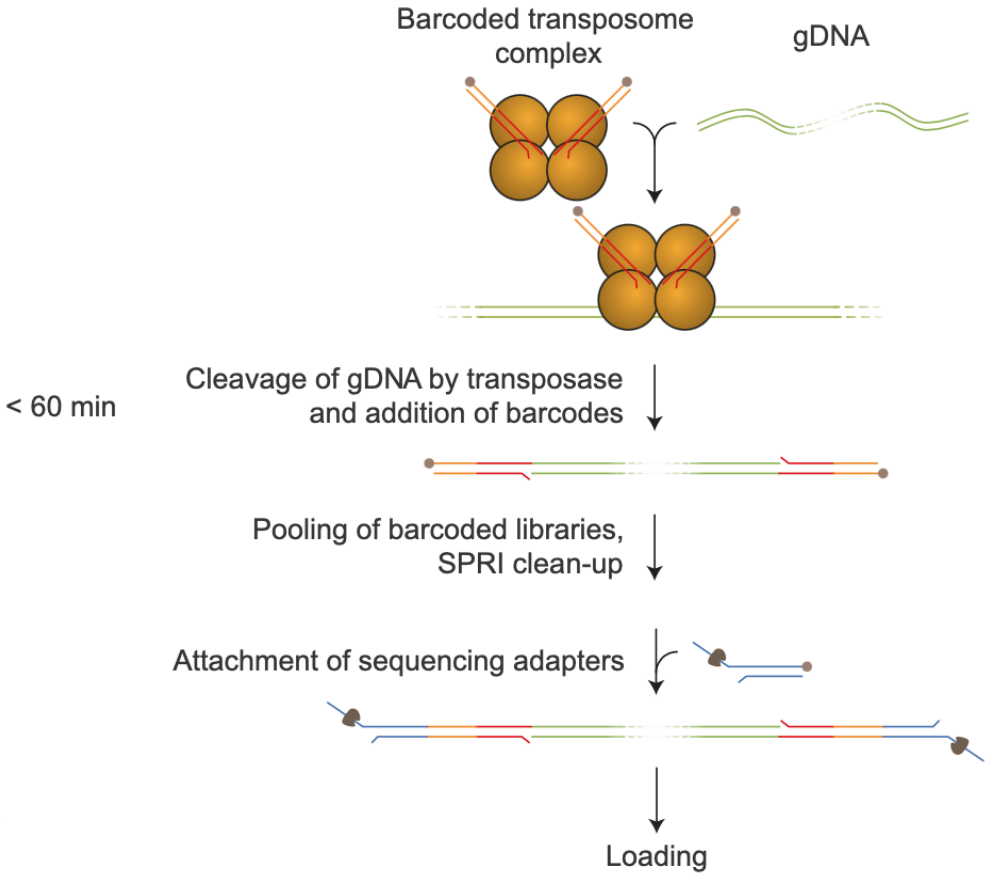
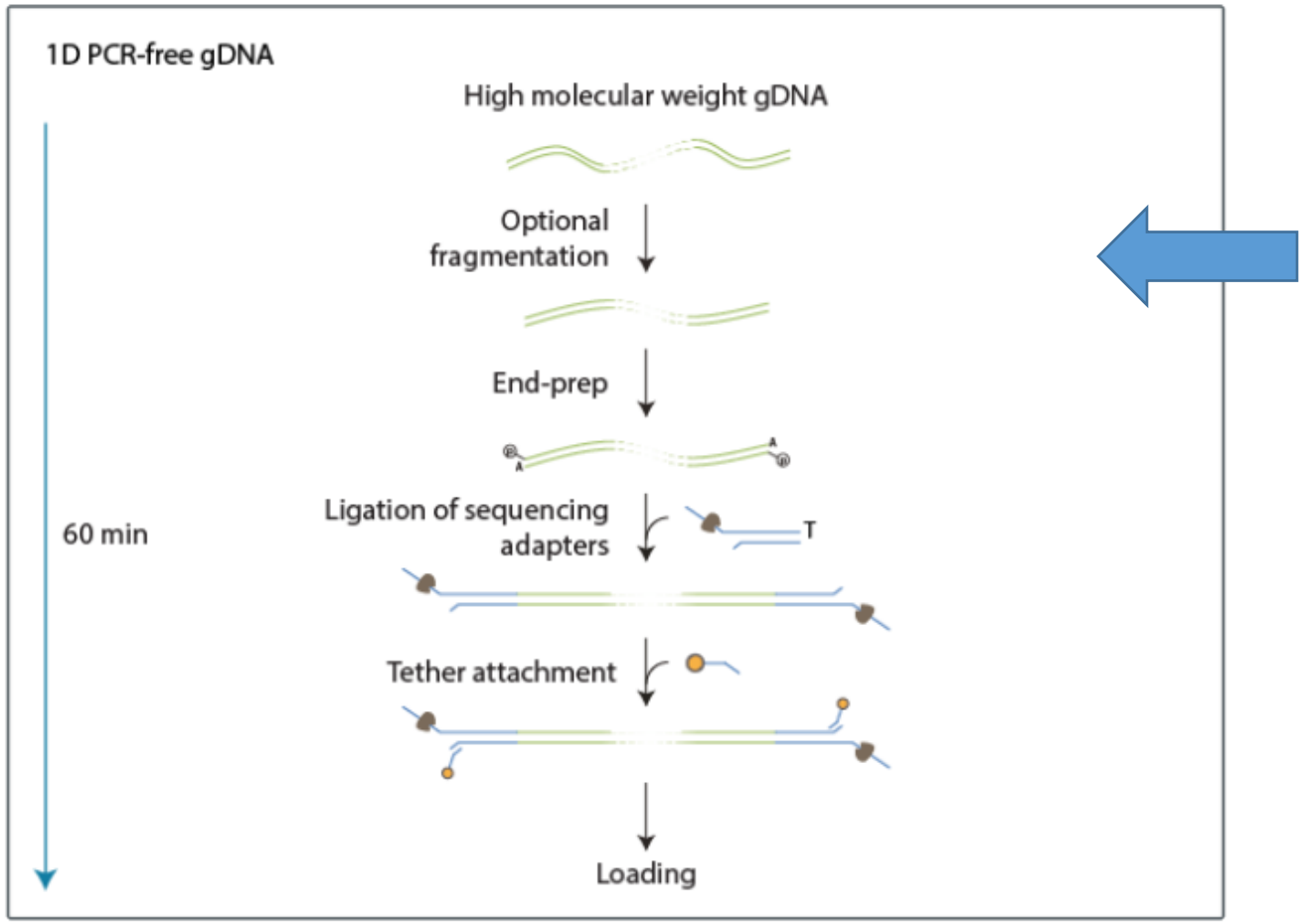
Assessing your DNA fragments

Purity	→	Nanodrop
Length	→	Tapestation or FemtoPULSE or gel
Amount	→	Qubit

Library construction



Rapid
Suited to fieldwork or ultralong sequencing



Ligation
Suited to maximising data yield

Image c/o Oxford Nanopore

Optional Fragmentation

Not recommended for some applications, e.g. short fragments, PCR products

Can be useful when some very long fragments are present but high throughput is priority

Options include G-TUBE (historically recommended by ONT), needle shearing, Megaruptor

Ligation vs Rapid

	Ligation	Rapid
Yield		
Time		
Cost		
Data quality		
Robustness		

Multiplexing

Make best use of a flow cell capacity by combining >1 sample together

Each sample is “labelled” with a known DNA sequence

Oxford Nanopore barcodes are longer than Illumina, at 24 bases

This allows confidence despite the higher per-base error rate, and makes use of Oxford Nanopore’s long reads

Component	Sequence
NB01	AAGAAAGTTGTCGGTGTCTTTGTG
NB02	TCGATTCCGTTTGTAGTCGTCTGT
NB03	GAGTCTTGTGTCCCAGTTACCAGG
NB04	TTCGGATTCTATCGTGTTTCCCTA
NB05	CTTGTCCAGGGTTTGTGTAACCTT
NB06	TTCTCGCAAAGGCAGAAAGTAGTC
NB07	GTGTTACCGTGGGAATGAATCCTT
NB08	TTCAGGGAACAAACCAAGTTACGT
NB09	AACTAGGCACAGCGAGTCTTGGTT
NB10	AAGCGTTGAAACCTTTGTCCTCTC
NB11	GTTTCATCTATCGGAGGGAATGGA
NB12	CAGGTAGAAAGAAGCAGAATCGGA

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Basecalling

Current or recently announced basecallers now allow:

- Real time methylation information
- Demultiplexing of samples
- Accurate sequencing of short fragments (>20bp)*

Oxford Nanopore Platforms and Products

Oxford Nanopore sequencing

MinION

Up to 50Gb

GridION

Up to 250Gb

PromethION

Up to 220Gb per flow cell
10.5Tb per instrument

Image c/o Oxford Nanopore

Images center and right c/o Oxford Nanopore

Why is it so small?

Other machines need to have cameras and lasers to take pictures of the DNA

They need to have moving parts to add chemicals to synthesise the DNA

The MinION measures electrical signals from DNA that is already present

MinION

Starter Pack — £900.00

Configure package

- 1x MinION Sequencing Device (MIN-101B)
- 1x Control Expansion Kit (EXP-CTL001)
- 1x Flow Cell Wash Kit (EXP-WSH004)
- 1x Flow Cell (R9.4.1) (FLO-MIN106D)

- or -

- 1x Flow Cell (R10.4.1) (FLO-MIN114)
- 1x Sequencing kits

Configure package

Enhanced — £2,936.00

Configure package

- 1x MinION Sequencing Device (MIN-101B)
- 1x Flow Cell Wash Kit (EXP-WSH004)
- 1x Control Expansion Kit (EXP-CTL001)
- 4x Flow Cell (R9.4.1) (FLO-MIN106D)

- or -

- 4x Flow Cell (R10.4.1) (FLO-MIN114)
- 1x Sequencing kits

Configure package

Don't forget cost of computer!

Prices c/o Oxford Nanopore store, taken 30/03/2022

Basic Starter Pack £4,410.00

Configure package

1x Flow Cell Wash Kit
EXP-WSH004

1x Control Expansion Kit
EXP-CTL001

1x MinION Mk1C
MIN-101C

6x Flow Cell (R9.4.1)
FLO-MIN106D

- or -

6x Flow Cell (R10.4.1)
FLO-MIN114

1x
Software Licence & Device warranty - Mk1C
SLW12M

1x Sequencing kits

Configure package

Enhanced Starter Pack £8,613.00

Configure package

1x Flow Cell Wash Kit
EXP-WSH004

1x Control Expansion Kit
EXP-CTL001

1x MinION Mk1C
MIN-101C

12x Flow Cell (R9.4.1)
FLO-MIN106D

- or -

12x Flow Cell (R10.4.1)
FLO-MIN114

1x
Software Licence & Device warranty - Mk1C
SLW12M

2x Sequencing kits

Configure package

CapEx £8,370.00

Configure package

1x MinION Mk1C
MIN-101C

1x
Software Licence & Device warranty - Mk1C
SLW12M

Configure package

Covid Starter Pack £8,168.00

Configure package

1x Flow Cell Wash Kit
EXP-WSH004

2x Rapid Barcoding Kit 96
SQK-RBK110.96

1x MinION Mk1C
MIN-101C

6x Flow Cell (R9.4.1)
FLO-MIN106D

1x
Software Licence & Device warranty - Mk1C
SLW12M

1x Sequencing kits

Configure package

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GridION

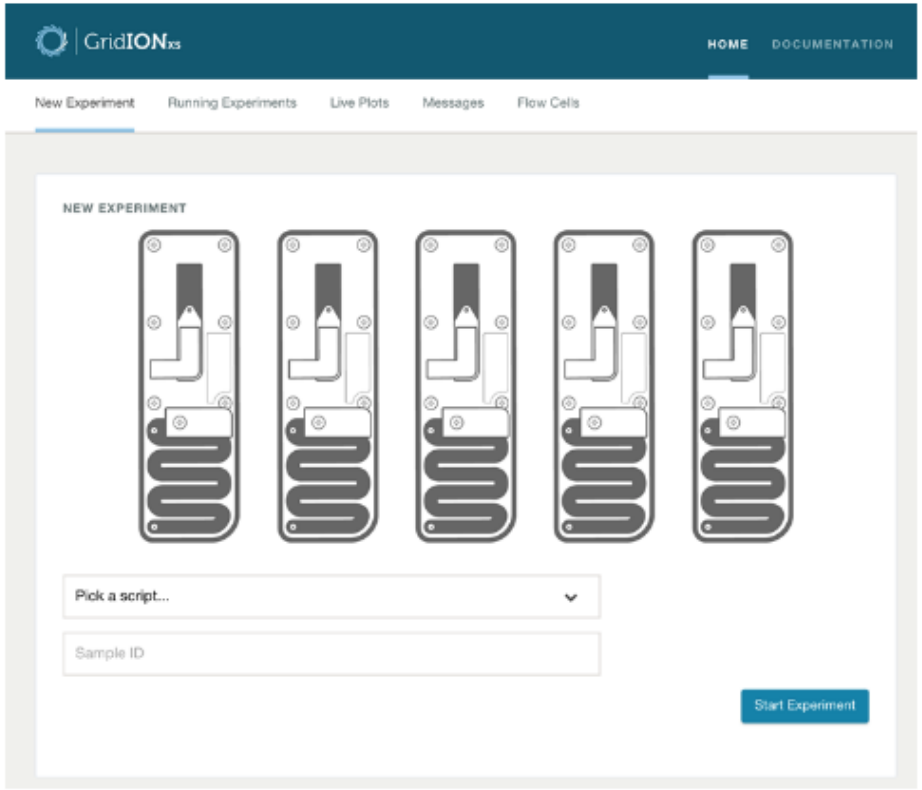

Image c/o Oxford Nanopore

GridION

Mk1 Starter Pack — £44,960.00

Configure package

1x
GridION Mk1 Sequencing Device - Loan Device (12 months)
GRD-X5B003

1x Assurance
ASSURANCE

1x Control Expansion Kit
EXP-CTL001

5x Flow Cell Wash Kit
EXP-WSH004

60x Flow Cell (R9.4.1)
FLO-MIN106D

- or -

60x Flow Cell (R10.4.1)
FLO-MIN114

1x
Software Licence & Device warranty - GridION
SLW12M-G

10x Sequencing kits

Configure package

Mk1 CapEX — £62,960.00

Configure package

1x Assurance
ASSURANCE

1x GridION Mk1 Sequencing Device
GRD-X5B003

1x
Software Licence & Device warranty - GridION
SLW12M-G

Configure package

Mk1 Covid Starter Pack — £72,821.00

Configure package

5x Flow Cell Wash Kit
EXP-WSH004

1x
GridION Mk1 Sequencing Device - Loan Device (12 months)
GRD-X5B003

1x Assurance
ASSURANCE

20x Rapid Barcoding Kit 96
SQK-RBK110.96

60x Flow Cell (R9.4.1)
FLO-MIN106D

1x
Software Licence & Device warranty - GridION
SLW12M-G

10x Sequencing kits

Configure package

NANOPORE Service Certified

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Image c/o Oxford Nanopore

PromethION

Image c/o Oxford Nanopore

48 flow cells

Each flowcell originally “50Gb” now maximum is 290Gb

Up to 14Tb of sequence

Rapidly evolving

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PromethION

24 (A100) Starter Pack — £202,518.00

Configure package

- 1x PromethION 24 Sequencing Unit — PRO-SEQ024
- 1x PromethION Data Acquisition Unit — PRO-PRCA100
- 1x PromethION Advanced Training — SUPP008
- 20x Flow Cell Wash Kit — EXP-WSH004
- 1x Control Expansion Kit — EXP-CTL001
- 48x PromethION Flow Cell Packs (R9.4.1) — FLO-PRO002

- or -

- 48x PromethION Flow Cell Packs (R10.4.1) — FLO-PRO114M
- 1x Software License & Device warranty - PromethION 24 A100 — SLW12M-P24A100
- 42x Sequencing kits

Configure package

48 (A100) Starter Pack — £279,071.00

Configure package

- 1x PromethION 48 Sequencing Unit — PRO-SEQ048
- 1x PromethION Data Acquisition Unit — PRO-PRCA100
- 1x PromethION Advanced Training — SUPP008
- 30x Flow Cell Wash Kit — EXP-WSH004
- 1x Control Expansion Kit — EXP-CTL001
- 72x PromethION Flow Cell Packs (R9.4.1) — FLO-PRO002

- or -

- 72x PromethION Flow Cell Packs (R10.4.1) — FLO-PRO114M
- 1x Software License & Device warranty - PromethION 48 A100 — SLW12M-P48A100
- 48x Sequencing kits

Configure package

24 (A100) CapEX — £306,003.00

Configure package

- 1x PromethION 24 Sequencing Unit — PRO-SEQ024
- 1x PromethION Data Acquisition Unit — PRO-PRCA100
- 1x PromethION Advanced Training — SUPP008
- 1x Software License & Device warranty - PromethION 24 A100 — SLW12M-P24A100

Configure package

48 (A100) CapEx — £405,001.00

Configure package

- 1x PromethION 48 Sequencing Unit — PRO-SEQ048
- 1x PromethION Data Acquisition Unit — PRO-PRCA100
- 1x PromethION Advanced Training — SUPP008
- 1x Software License & Device warranty - PromethION 48 A100 — SLW12M-P48A100

Configure package

Image c/o Oxford Nanopore

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P2

A “mini”

PromethION

Also as “solo” model – plugs into GridION or computer

Image c/o Oxford Nanopore

Flongle

Image c/o Oxford Nanopore

Adapts MinION to take cheap, low throughput flow cells

Q Line

SO 9001:2015 certified manufacturing process

12 months guaranteed supported software & consumables

Clearly mapped out upgrades

Same price as standard devices & consumables

Allows in house validation of assays for long term use

Q Line

Ligation (& barcoding) kit
Rapid (& barcoding) kit
GridION

Recommended for environmental & veterinary diagnostics, forensics, and clinical research.

Not licenced for human diagnostic use

Where has Oxford Nanopore been used?

MUSE/Science Museum of Trento

The MinION device can sequence small genomes, such as those of bacteria and viruses, displaying the results as they are generated.

http://www.nature.com/news/pint-sized-dna-sequencer-impresses-first-users-1.17483

Image c/o NASA, USA

Instead of shipping samples to fully-equipped laboratories for analysis, the MinION device can send sample data via a USB. This information can then be sequenced and analysed within 24 hours, instead of the weeks it usually takes. Photograph: Tommy Trenchard/EMLabs

Image from The Guardian Online
https://www.theguardian.com/science/2016/feb/03/from-ebola-to-zika-tiny-mobile-lab-gives-real-time-dna-data-on-outbreaks

SARS-CoV-2

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Concluding thoughts

Oxford Nanopore sequencing

Advantages	Disadvantages
Portable	Can require highly pure DNA
Rapid	Can require large amounts of input DNA
Low start up costs	Less supported analysis
Native DNA sequencing	
Long read	
Multiplexing options	
Fleet of sequencers	

Floatation

Oxford Nanopore Technologies floated on the London Stock Exchange

Currently valued at ~£2.2 billion ($2.72 billion – for comparison Illumina ~$31.6 billion)

What does this mean for your science?

- very little
- perhaps greater visibility of how the company is performing

Additional technologies

华大基因 BGI

PACBIO®

bionano GENOMICS

10x GENOMICS

ion torrent by life technologies

Base4

genia

press.office@sanger.ac.uk

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- All scripts/software = open source
- Slides & links available on your course webpage
- Questions?

 kj6@sanger.ac.uk

 @kim_judge_

Analysis session

Objective

Assemble bacterial data generated using the latest Oxford Nanopore Chemistry (as used this morning)

Check completeness of assembly

Investigate downsampling data to get the best – or fastest – assembly

Each genome can have many assemblies!

1. Install minimap2 and miniasm

Search for the website

https://github.com/lh3/miniasm

Copy the commands into your terminal

git clone https://github.com/lh3/minimap2 && (cd minimap2 && make)

git clone https://github.com/lh3/miniasm && (cd miniasm && make)

2. Run the overlap

```
minimap2/minimap2 -x ava-ont  /path/to/barcode1.fastq /path/to/barcode1.fastq > barcode1.paf
```

3. Run the assembly

```
miniasm/miniasm -f /path/to/barcode1.fastq barcode1.paf > barcode1.gfa
```

Minimap2 & miniasm will work with compressed (zipped) data files, so you can try running:

```
minimap2/minimap2 -x ava-ont /path/to/reads.fq /path/to/reads.fq | gzip -1 > reads.paf.gz
miniasm/miniasm -f reads.fq reads.paf.gz > reads.gfa
```

4. Assess the assembly

Process the assembly to get fasta format

awk '$1=="S" {print ">"$2"\n"$3}' sample1.gfa **>** sample1.fa

Then Try:

head
wc - l
tail
ls -l
assembly-stats

5. Try on other samples

Use chopper to filter the reads to downsample, or "throw away" data

What happens when you downsample too much?

What happens if you don't downsample?

Use chopper - h or - - help to see options

To use chopper:

cat /path/to/barcode2.fastq | chopper –q 7 –l 500 > barcode2_filtered.fastq

Round up and final questions