Nanopore sequencing
High molecular weight DNA isolations
Hi-C

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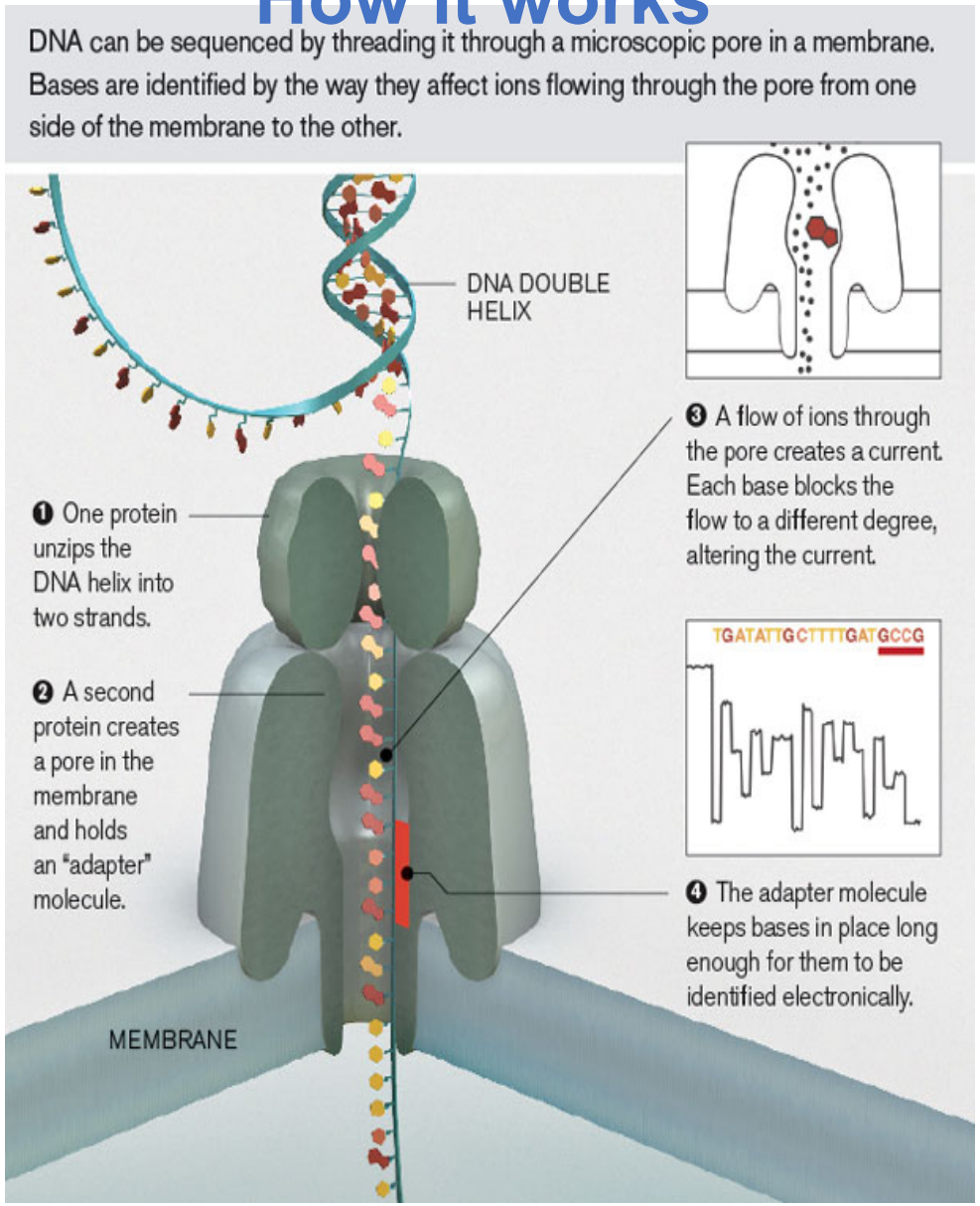
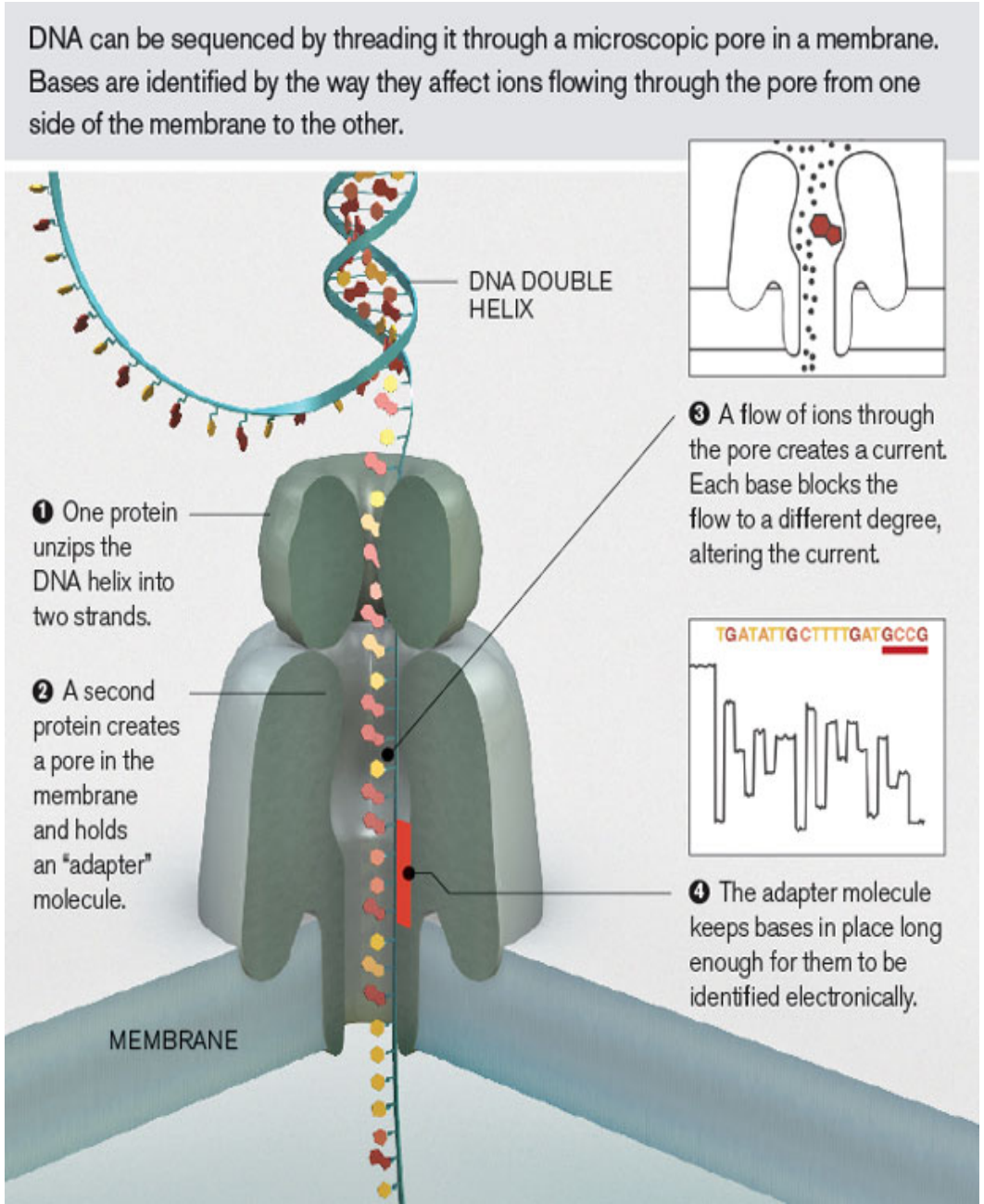
Oxford nanopore sequencing
How it works



Oxford nanopore sequencing



Long read sequencing

In theory read length is limited by the length of input DNA

Simpler workflow
Robust equipment
Sequencer hardware is electronics highly scalable

Lower cost per bp sequence

Higher rates of not-fully random errors

ONT sequencing platforms available in the DNA Tech Core

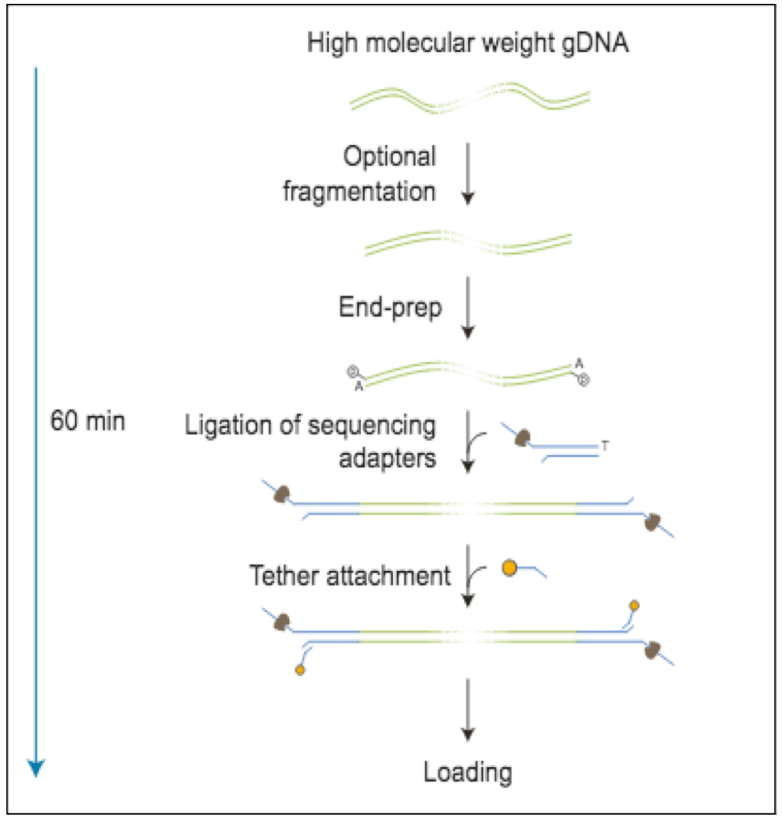
MinION - portable sequencer

Low throughput
Can run single flowcell at a time
2-10 Gbp yield per flowcell

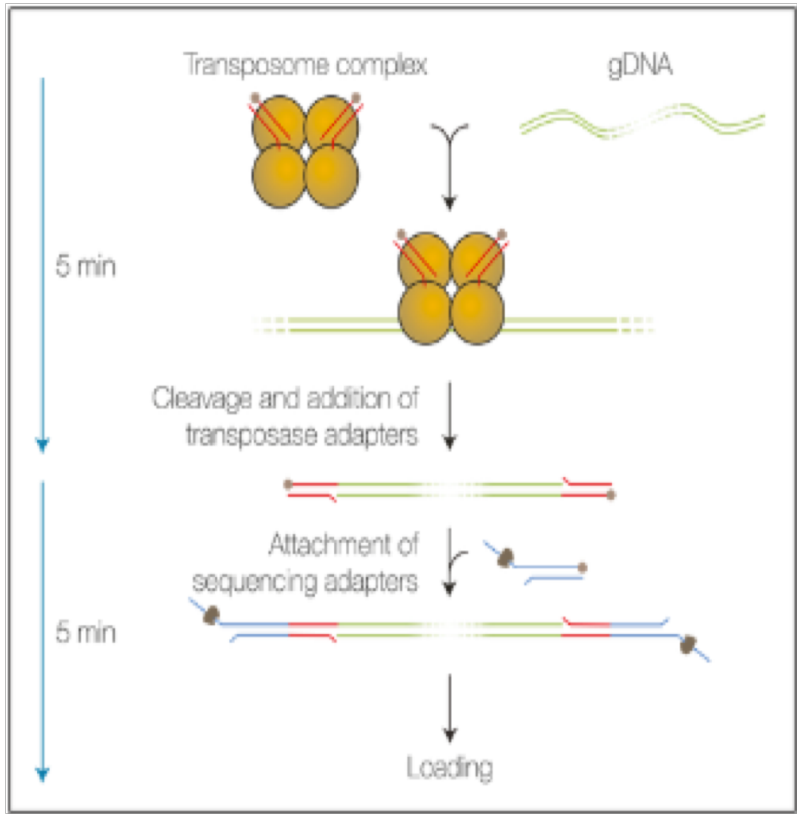
PromethION

High throughput
Can run up to to 24 flowcells at a time
20-90 Gbp yield per flowcell

Sample preparation kits



Ligation Sequencing kit



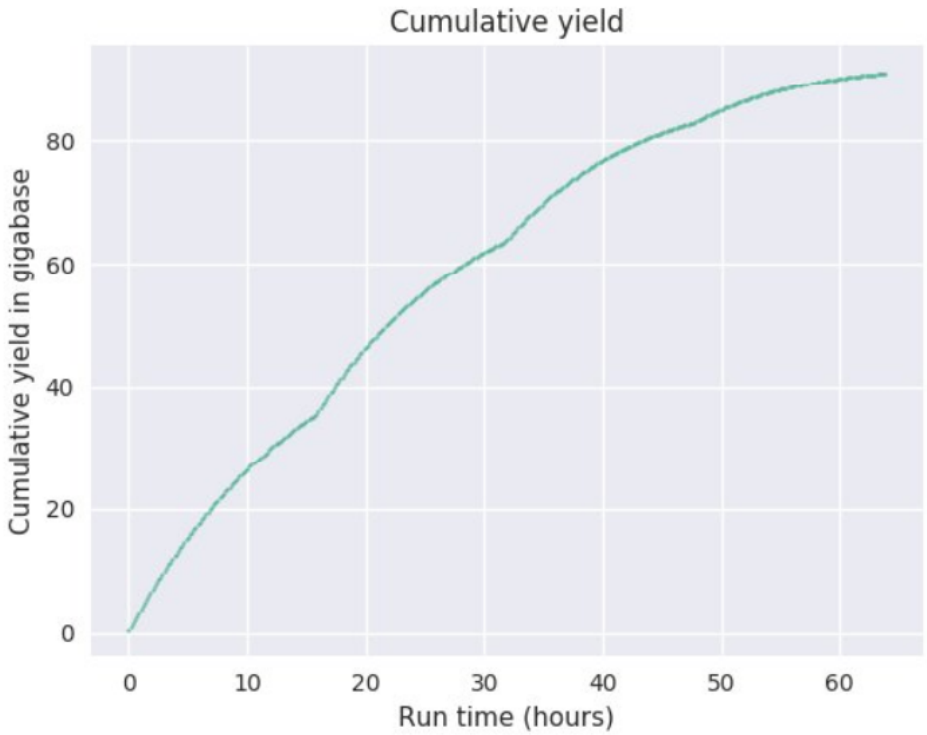
Rapid sequencing kit

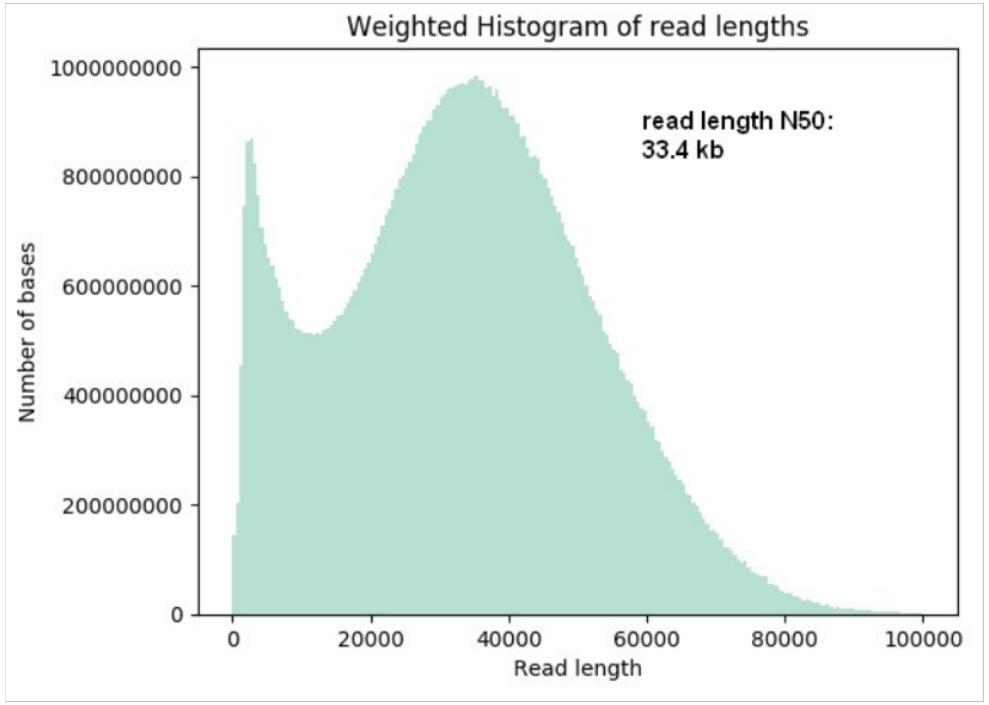
Different types of workflows available for DNA sequencing with ONT platforms

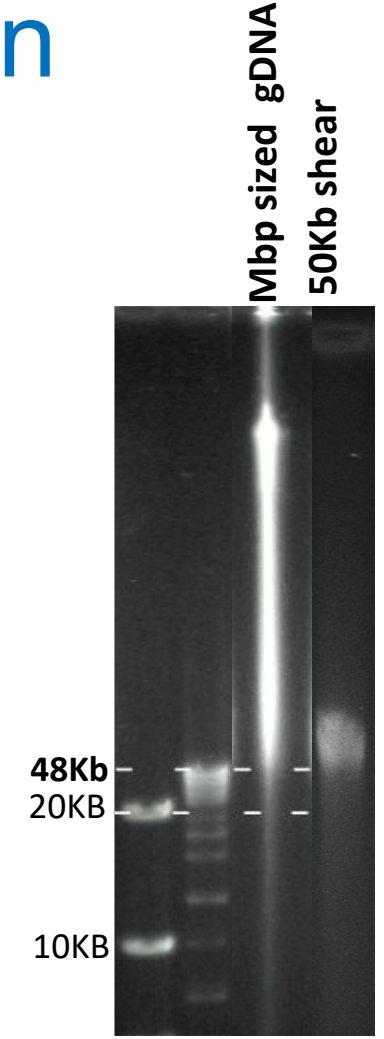
Super long read sequencing – HMW DNA > 50Kb in length, PromethION flowcell yield range from 20 Gbp to 90 Gbp, read length N50 ~30 Kb, can generate reads >200 kb in length

Long read sequencing – DNA 5Kb to 20Kb in fragment length, yield per flowcells can range from 20 Gb -100 Gbp, read length N50 10Kb

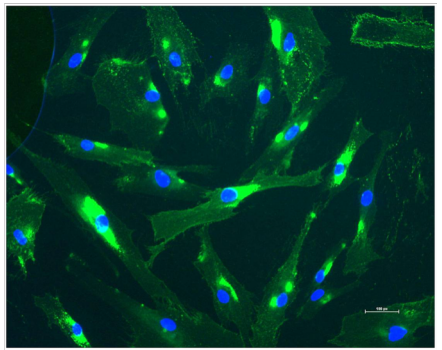
Example of a good PromethION run



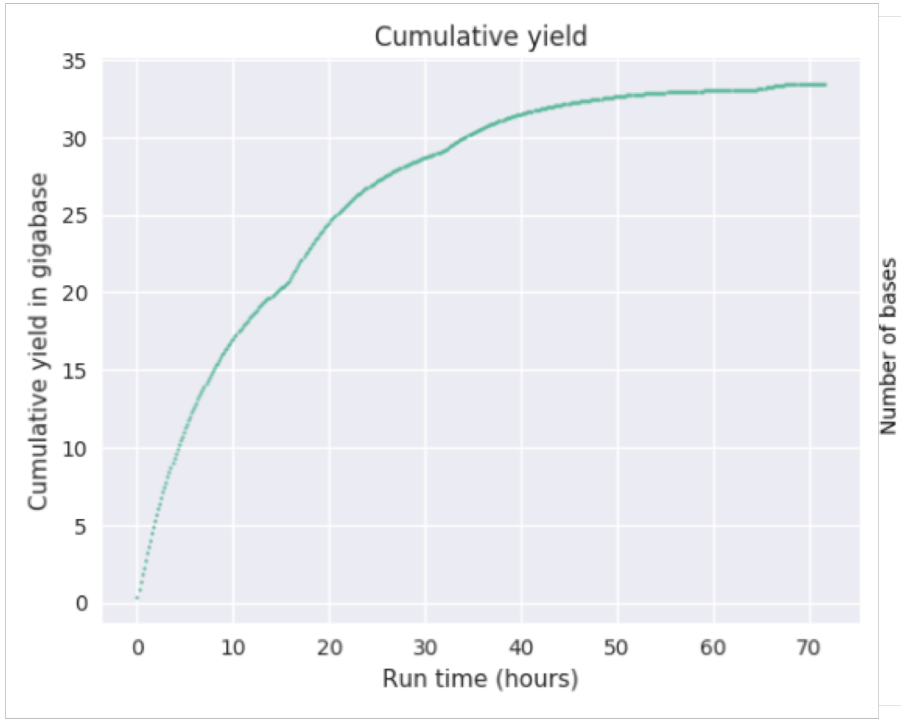


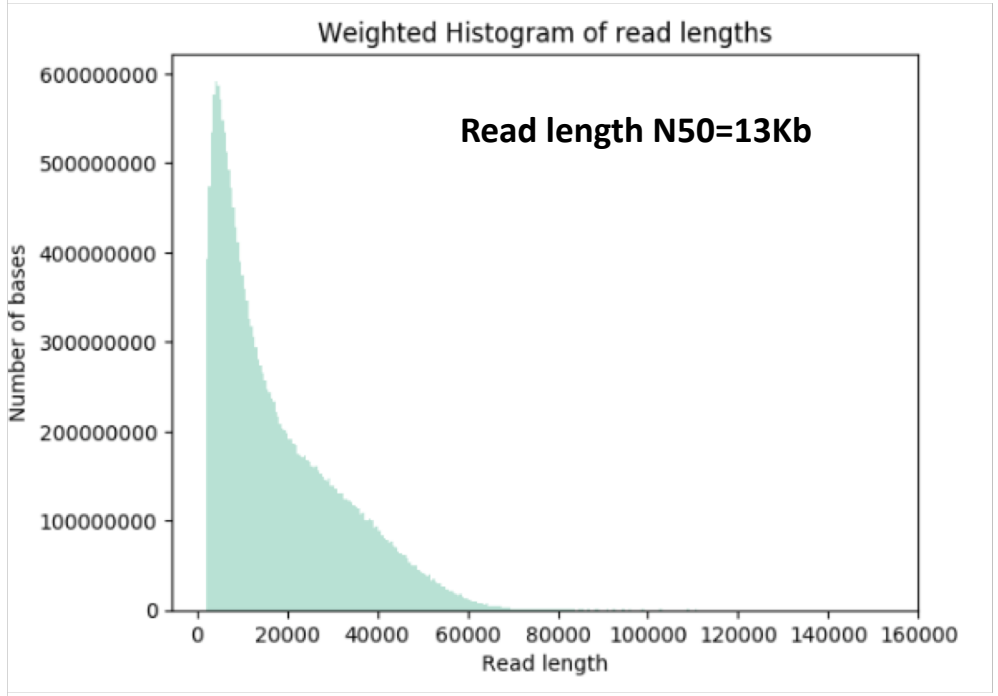


Good quality DNA isolated from cultured mammalian cell lines or blood sample can generate up to 90Gbp of data with read length N50 of 30Kb



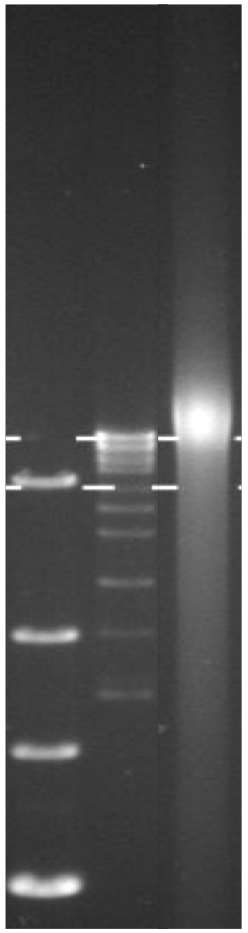
Example of an OK PromethION run





Beautiful killifish but so so DNA

48Kb



Killifish DNA

Different types of workflows available for DNA sequencing with ONT platforms

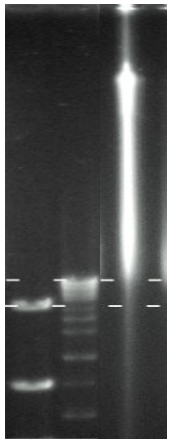
Super long read sequencing – HMW DNA > 50Kb in length, PromethION flowcell yield range from 20 Gbp to 90 Gbp, N50 can reach up to 33 Kb, longest reads >200 kb in length

Long read sequencing – DNA 5Kb to 20Kb in fragment length, yield per flowcells can range from 20 Gb -100 Gbp, read length N50 10Kb

Ultra-long-read DNA sequencing

Ultra-long-read DNA sequencing

Mbp sized gDNA
15-20µg



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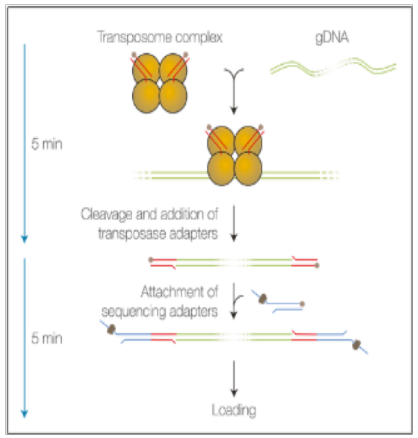
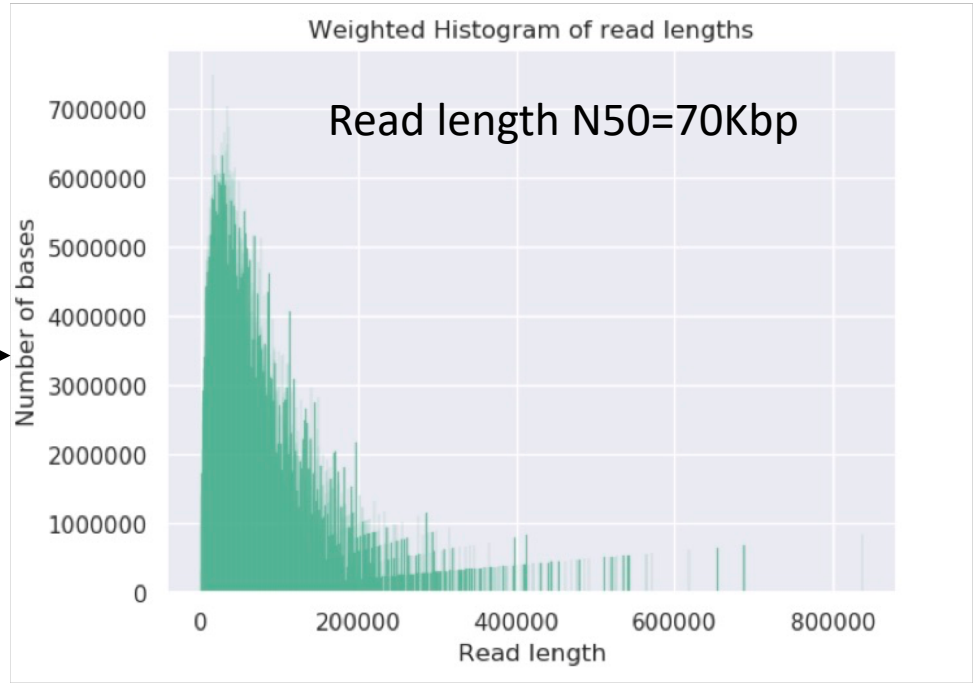


Rapid chemistry

+

MinION

→



Available only on MinION, lower yields (1Gb – 2Gb)

Factors influencing sequencing yield and run matrices

Sample quality

samples should be free of any contaminants such as salt, EDTA, protein, organic solvents

DNA damage will negatively influence the run

Certain species perform worse than other



Is there something fishy with bird DNA?

cnidaria, marine life, birds

Nanopore is working on updated protocols for these difficult samples

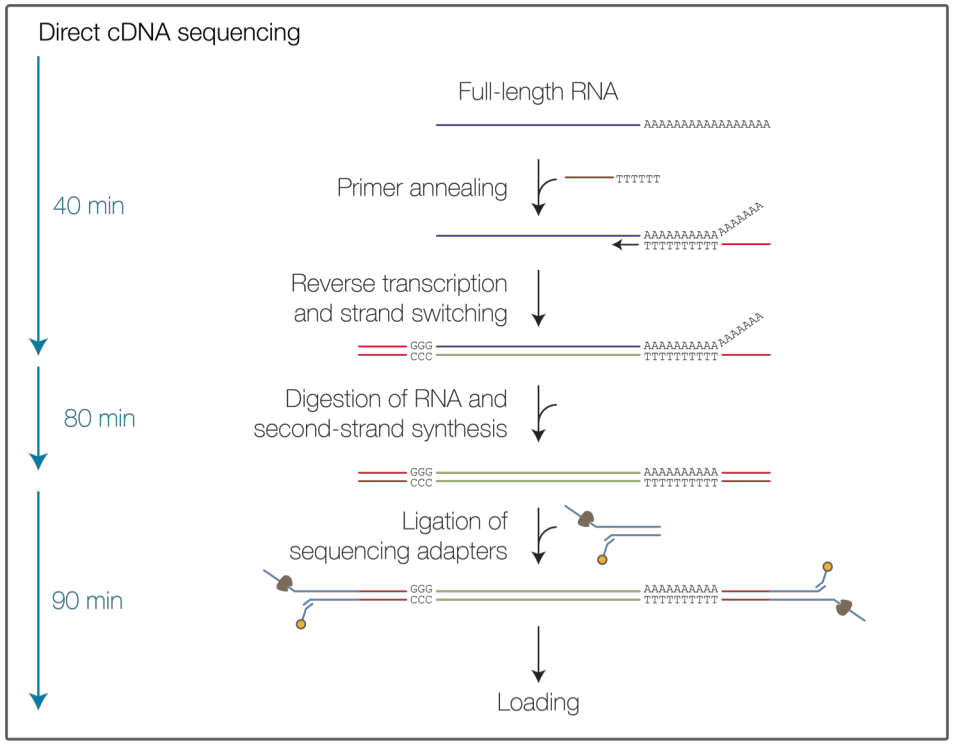
Flowcell quality

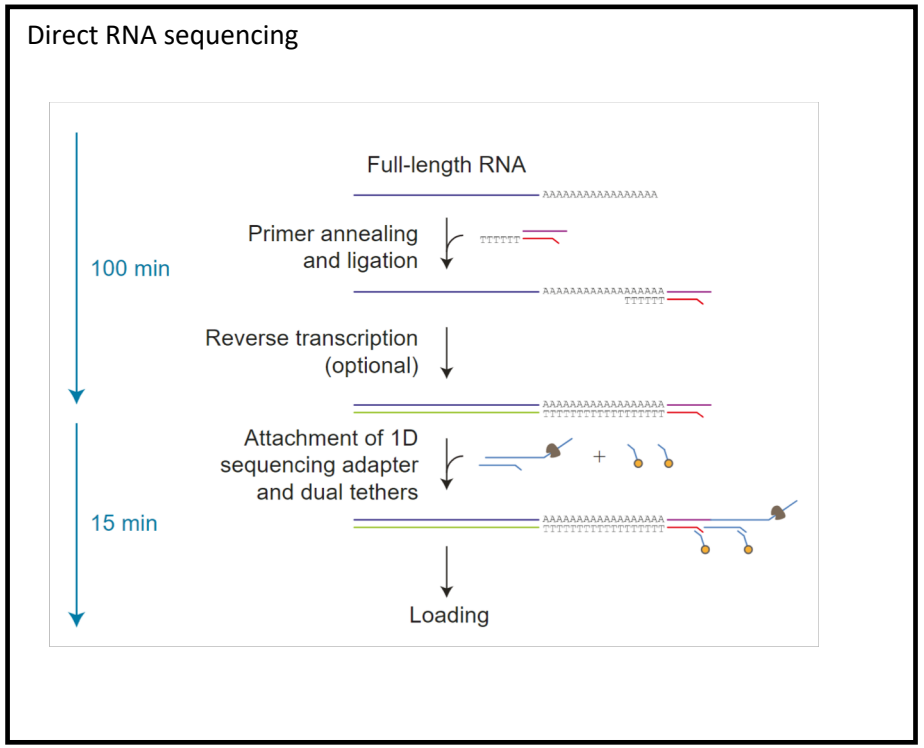
Number of active pores on a PromethION flowcell can range from 5000 to >9000

Input DNA requirement for nanopore sequencing

- Good quality, high molecular weigh DNA >50Kb in length
- Free of contaminants such as polysaccharides, proteins, salts, etc
- Nanodrop ratio of 260/280=1.8 260/230=2.0
- >5µg input

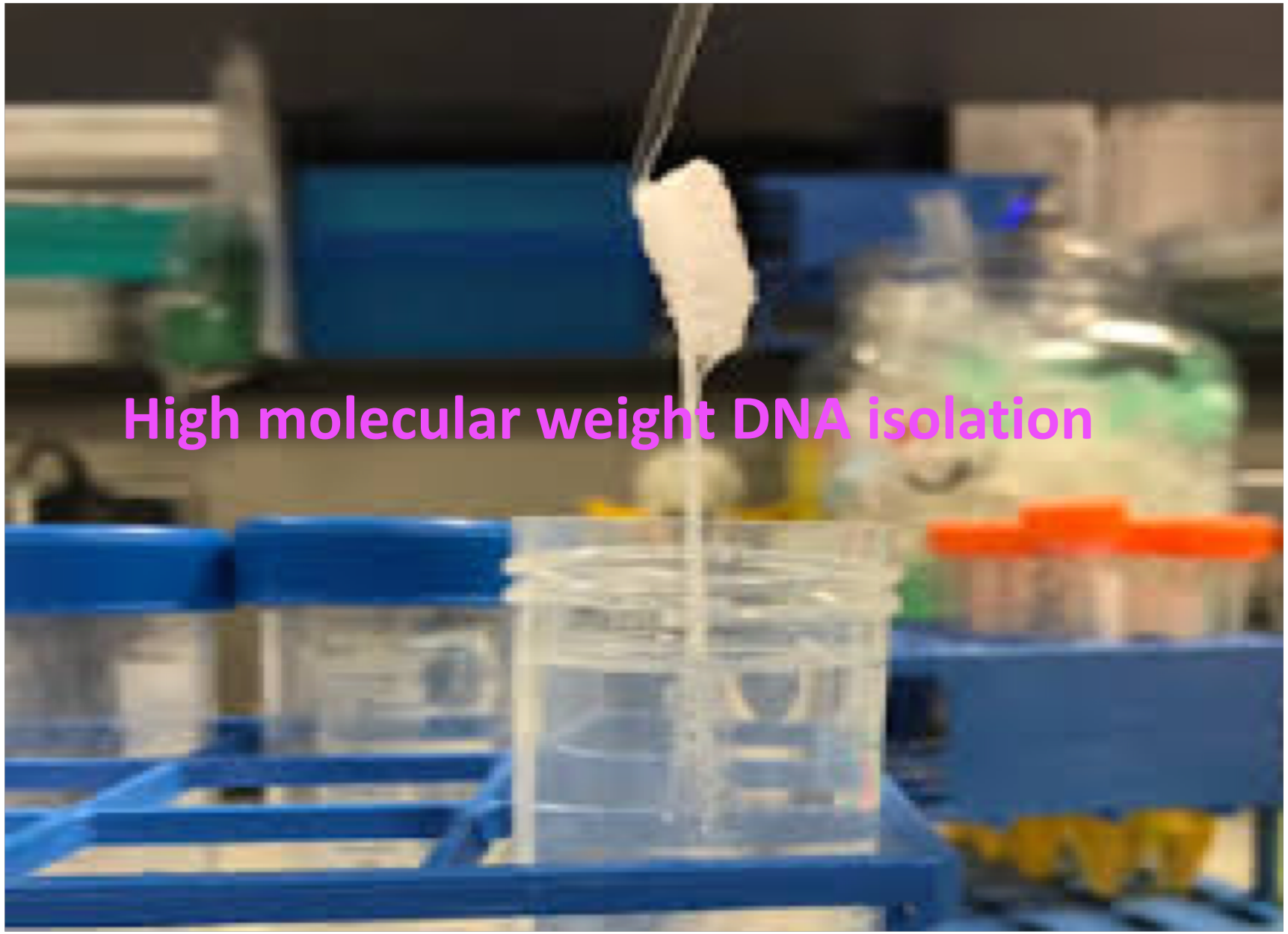
cDNA and direct RNA sequencing





100ng -2µg total RNA
> 50 million reads

Needs >500ng poly A RNA (~50µg of total RNA? or more)
Yield 1-4 Gbp
Only on MinION
Can detect RNA modifications

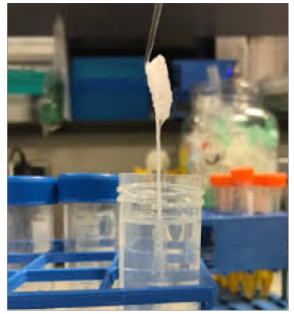

High molecular weight DNA isolation

High molecular weight DNA isolation

Spin column based methods not suitable

Animal cells and tissue Going back to old school, modified Sambrook and Russell protocol

Protein salting out



Qick et al, protocols.io

Plant tissues

CTAB

Nuclei enrichment

Starting material for HMW DNA extractions

- Cultured cell lines
- Whole blood or white blood cells
- Soft cellular tissue
- Insect pupae
- Young leaves, etiolated tissues

Starting material for HMW DNA extractions

- Cultured cell lines
- Whole blood or white blood cells

Works very well!

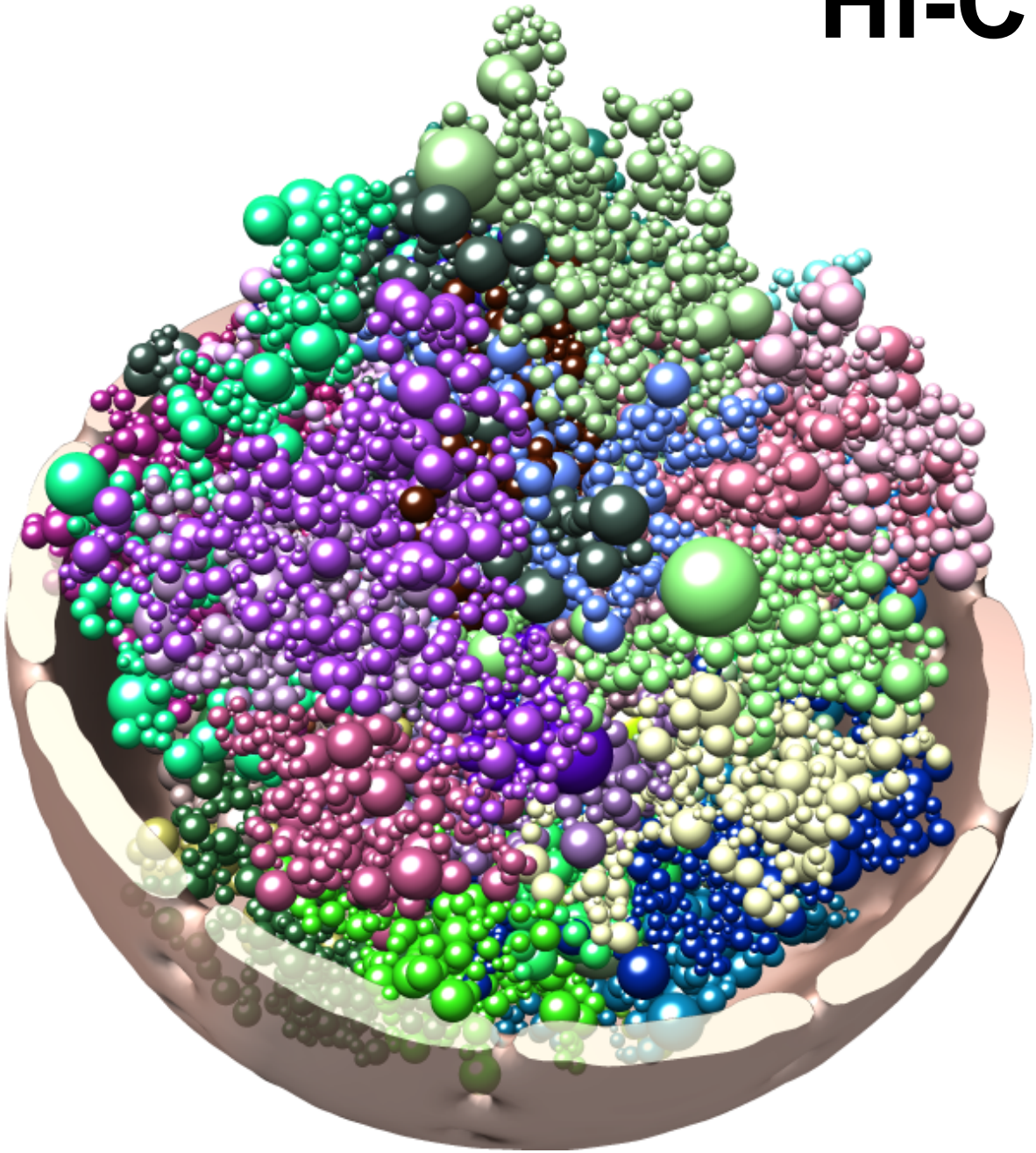
- Soft cellular tissue
- Insect pupae
- Young leaves, etiolated tissues

Proper tissue preservation is very important !!!

- Cultured cell line – trypsinize the cells, wash with PBS, remove PBS, flash freeze in liquid nitrogen. Store at -80 and transport on dry ice
- Blood – Use appropriate anticoagulant (EDTA or ACD)
- Soft tissue – flash freeze right after harvesting, store at -80 and transport on dry ice

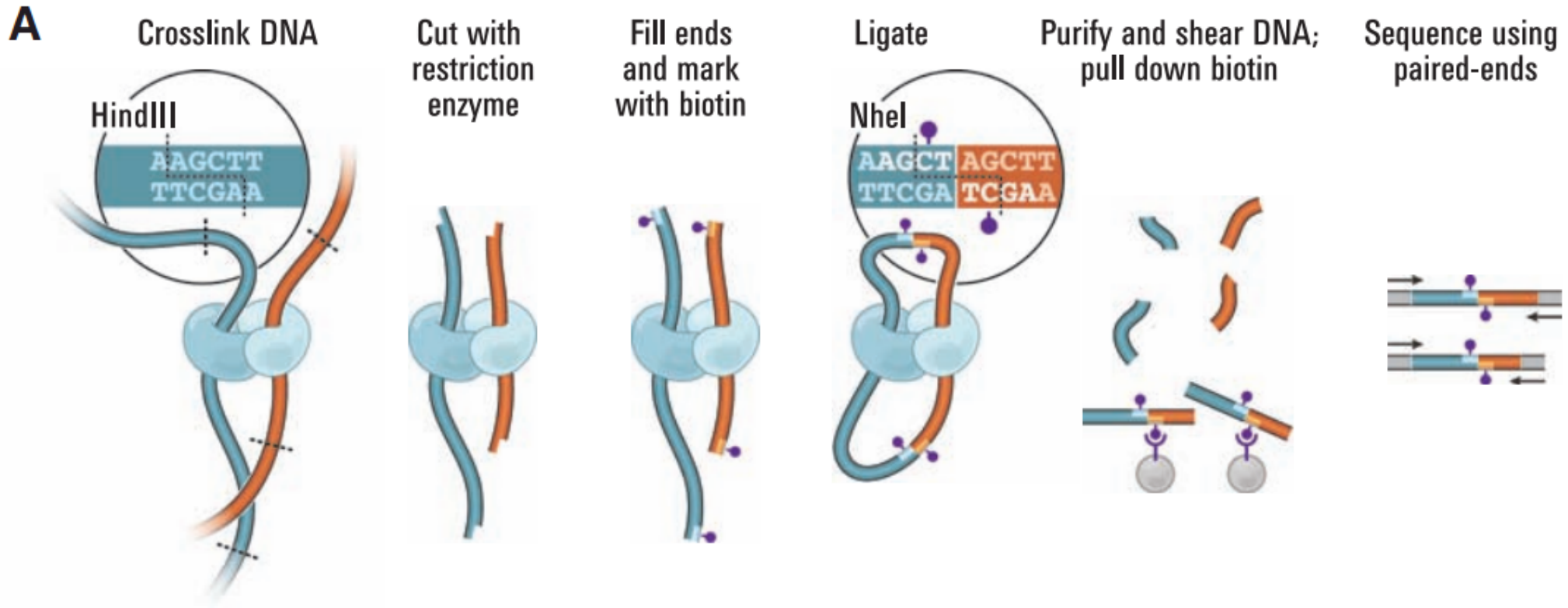
- Lyophilized tissue, tissue in RNA later can also work but fresh or flash frozen tissue is preferable. Ethanol preservation is not recommended
- Avoid freeze thaw cycles, remove guts or other source of microbial contamination

Hi-C



- Chromosome scale scaffolding
- Long – range interactions

Hi-C sample preparation



Lieberman-Aiden 2010

Input sample requirements for Hi-C

Cultured cell: 0.5million -1 million per reaction
Fresh frozen tissue: 25mg to 50 mg per reaction, soft cellular tissue such as muscle, heart, lung is preferable. Liver not accepted.
Fresh young leaves: 5g to 10g
Proper tissue preservation is important!!

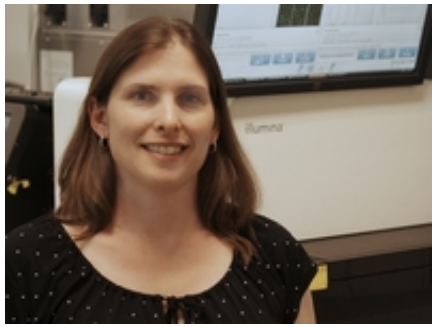
It is multi-step protocol and involves multiple QC steps to ensure that there is enough ligation products

100M -200M PE reads/Gbp of genome

Analysis: Proprietary software: HiRise, Proximo
open source alternatives

Thank you!

Lutz Froenicke
Core Director

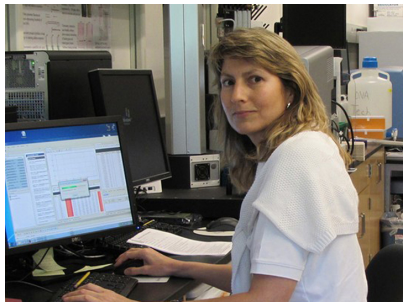


Emily Kumimoto
library preps

Oanh Nguyen
PacBio Seq.

Diana Burkardt-Waco
10X Genomics, HiSeq

Siranoosh Ashtari
all Illumina Seq.



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Miseq, Bead Array, Fludigm

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HMW DNA , Nanopore, Hi-C