

Advanced Applications of Next Generation Sequencing in Food Safety Scientific Programme

22 – 25 January 2024



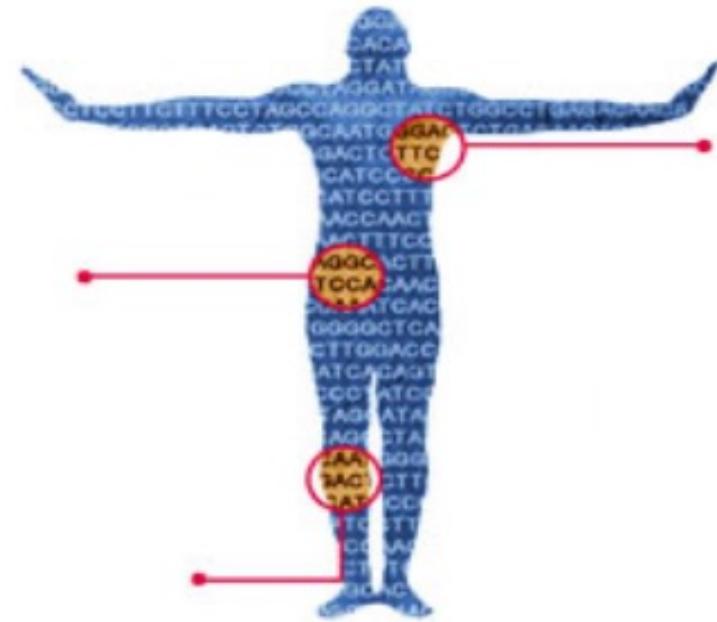
Whole Genome Sequencing Introductory talk and experimental set up



Background/Introduction

"A prerequisite to understanding the complete biology of an organism is the determination of its entire genome sequence"

Fleischmann *et al.* 1995



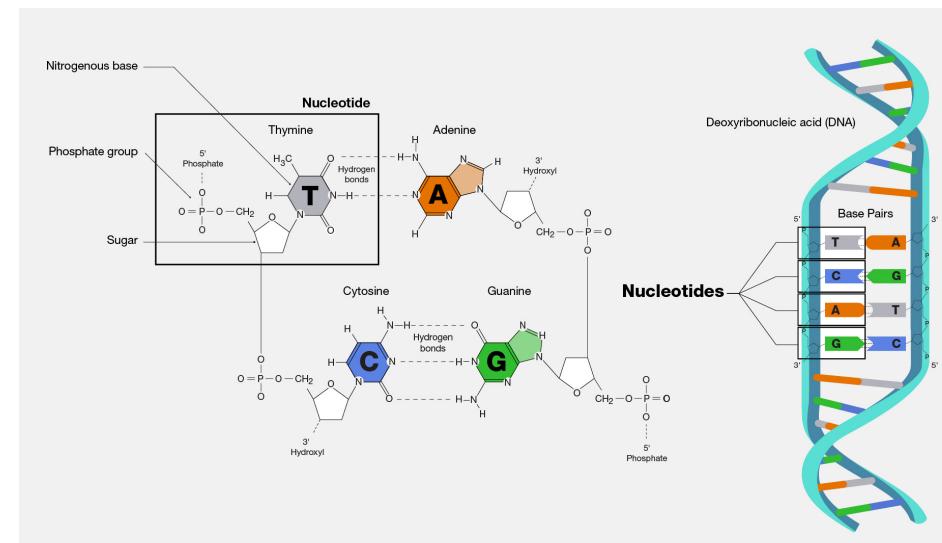
Background

DNA / RNA Sequencing:

- DNA & RNA strands consist of different combinations of four nucleotide bases: A, T (U in RNA), G and C.
- DNA = genetic code of life.
- RNA= messenger molecule that controls protein synthesis based on the DNA code.
- Sequencing can answer numerous biological questions:
 - Pathogen identity
 - Genetic disease
 - Evolution of an organism



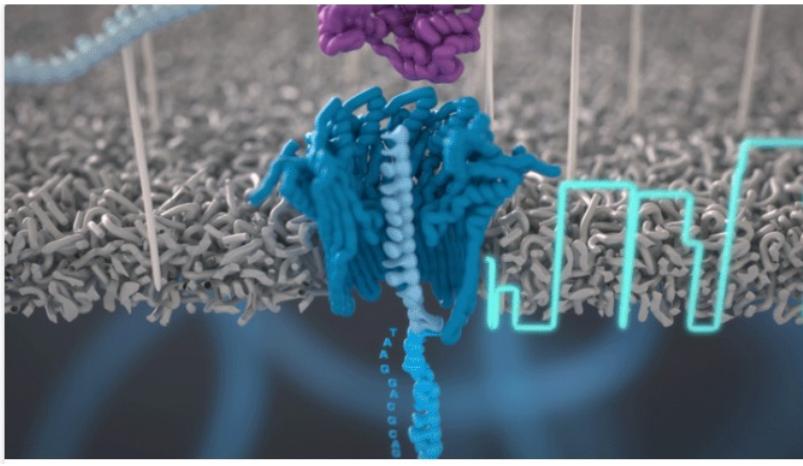
The Flongle Flow Cell can generate up to 2.8 Gb of data enabling direct, real-time DNA & cDNA sequencing on smaller, single-use flow cells.



Introduction

Oxford Nanopore Sequencing devices:

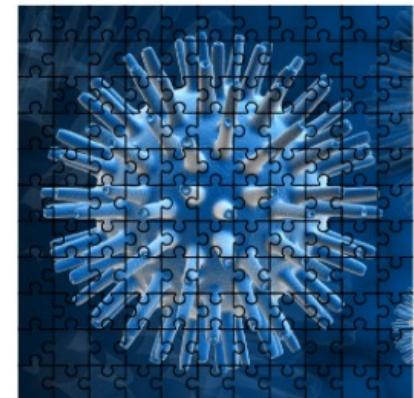
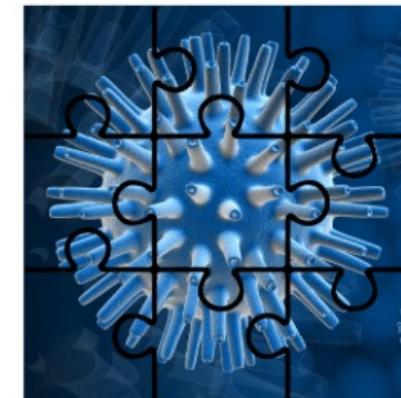
- Use **flow cells** with tiny holes (**nanopores**) enclosed in an **electro-resistant membrane**.
- Every nanopore is linked to an individual electrode which is connected to a channel and sensor chip (measures the **electric current** flowing through the nanopore).
- This **current is disrupted** when a **molecule/nucleotide base (A, T (or U), C, or G) passes through the nanopore** and produces a characteristic **signal**.
- **Basecalling algorithms** are used to interpret the **signal** and hence ascertain the **DNA (or RNA) sequence** in real time/ rapid insight.



Introduction

The power of long reads:

- Traditional methods can only sequence **short fragments of DNA**; must then be **reassembled**.
- **Difficult** to sequence repetitive regions for accurate genome assemblies due to:
 - **Gaps**
 - Ability to resolve **large structural variations**
 - Ability to **differentiate isoforms**
- **Nanopore sequencing circumvents these challenges.**
- The only limitation is the length of the DNA fragment presented to the pore. The use of a transposase instead of PCR amplification also **eliminates PCR bias and allows for base /epigenetic modification identification** (methylation, acetylation, phosphorylation) in addition to nucleotide sequencing.



You can think of this like trying to complete two jigsaws of the same photograph – one with significantly larger pieces than the other. A jigsaw with only 9 pieces is much easier to assemble than one with 900.

Introduction

Whole Genome Sequencing (WGS):

- Complete analysis of an organism's genome (telomere-to-telomere).
- 1 Bacterium at a time.
- Wizard® Genomic DNA Purification Kit = To extract long fragments of gDNA
- Base pair (bp) depends on size of bacterium in question.
- Oxford Nanopore Rapid Sequencing kit V14 (SQK-RAD114) = To sequence and produce long reads from the long fragments.
- More readily available now due to comprehensive and cost-effective modern sequencing techniques.

Inspiration for whole-genome sequencing

Discover more about applying nanopore whole-genome sequencing to your organism and genomic variants of interest.

Research areas



Human genetics



Cancer research



Clinical research



Population genomics



Microbiology



Animal research

Investigations



SNVs

Structural variation

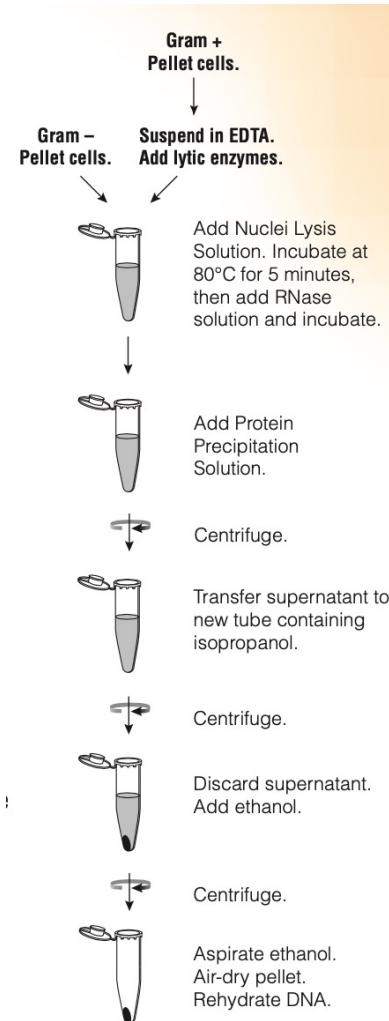
Phasing

Epigenetics

Assembly

Chromatin conformation

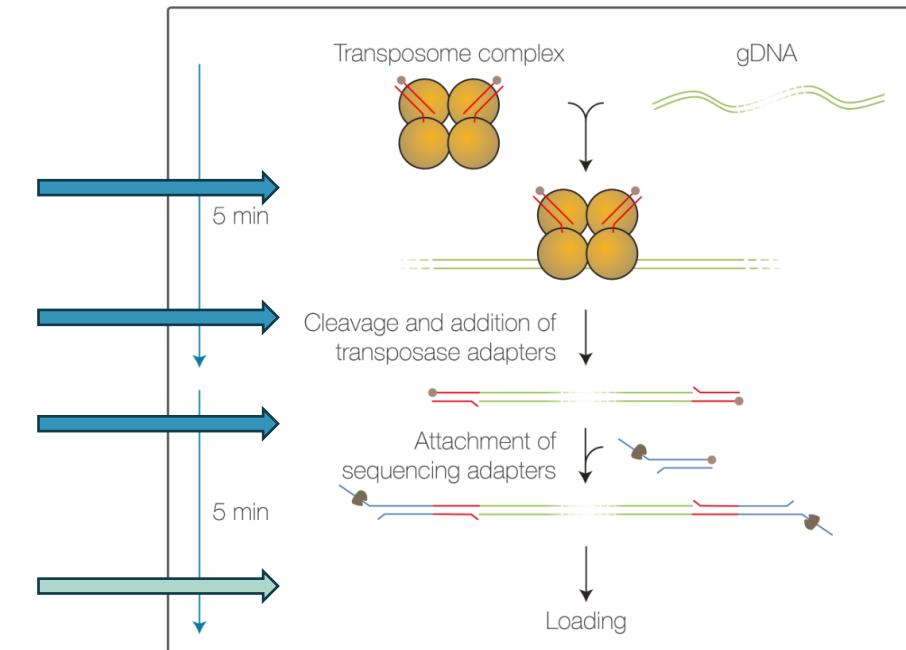
WGS process - Wizard® Genomic DNA Purification Kit



WGS process - WGS Library Preparation

Oxford Nanopore Rapid Sequencing kit V14 (SQK-RAD114)

Reagent	Step	Function
Fragmentation Mix (FRA)	WGS Library preparation	To fragment the DNA – transposase cleaves template molecules
Adapter Buffer (ADB)	WGS Library preparation	Transposase then attaches tag to cleaved ends
Rapid Adapter (RA)	WGS Library preparation	Rapid Sequencing Adapters are then added to the tagged ends for sequencing on a flow cell
Library Beads (LIB)/ Library Solution (LIS)	To prepared Library	Add to prepared library to load into the flow cell
Sequencing Buffer (SB)	To prepared Library	
Flow Cell Flush (FCF)	Flow cell	To prime the flow cell before loading the prepared library for sequencing
Flow Cell Tether (FCT)	Flow cell	

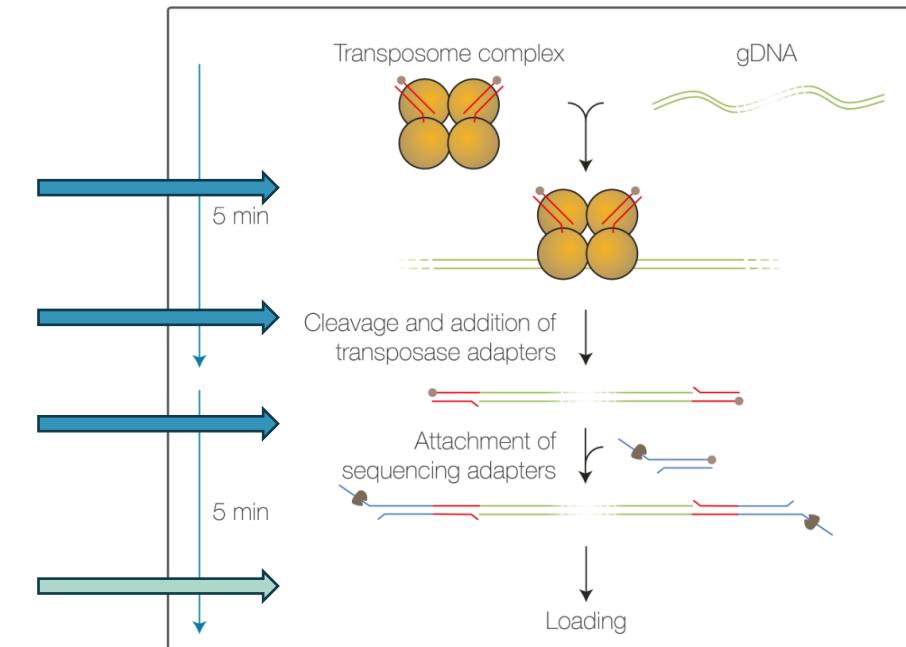


1 sample at a time

WGS process - WGS Library Preparation

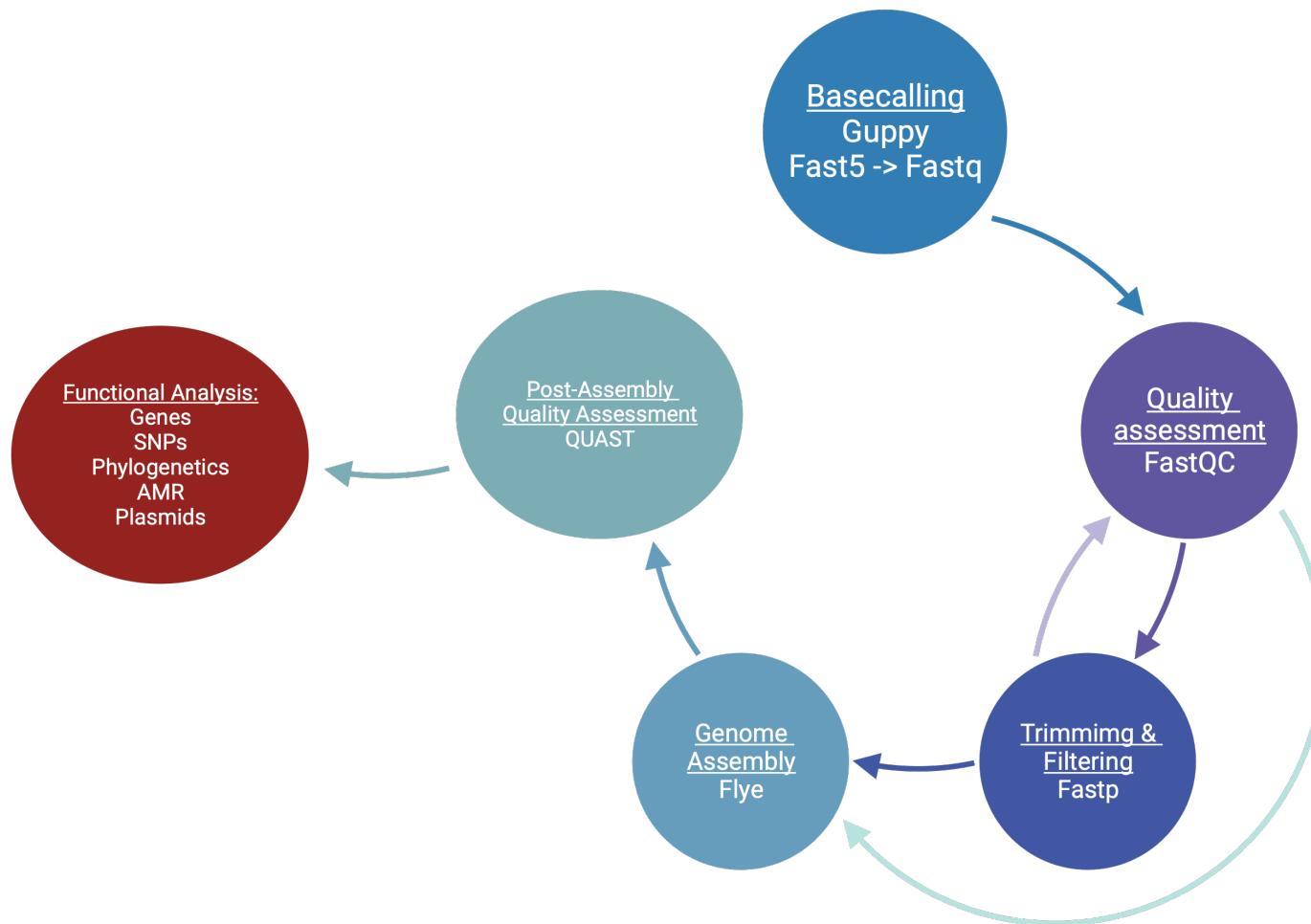
Oxford Nanopore Rapid Barcoding Kit 96 V14 (SQK-RBK114.96)

Reagent	Step	Function
Rapid Barcodes	WGS Library preparation	To fragment the DNA + to attach barcode tags to cleaved ends of DNA
Adapter Buffer (ADB)	WGS Library preparation	Transposase then attaches tag to cleaved ends
Rapid Adapter (RA)	WGS Library preparation	Rapid Sequencing Adapters are then added to the tagged ends for sequencing on a flow cell
Library Beads (LIB)/ Library Solution (LIS)	To prepared Library	Add to prepared library to load into the flow cell
Sequencing Buffer (SB)	To prepared Library	
Flow Cell Flush (FCF)	Flow cell	To prime the flow cell before loading the prepared library for sequencing
Flow Cell Tether (FCT)	Flow cell	



Multiple samples at a time

Bioinformatics Pipeline



Thank you

- To the CFS labs of Stellenbosch University and UCD

