Whole Genome Sequencing

by Nanopore Long Read Technology

Introduction

Oxford Nanopore Technologies (ONT) real-time long-read sequencing technology and library preparation kits allow to sequence native DNA eliminating PCR bias from the data. The technology feeds a single-stranded DNA molecule through a protein nanopore and measures changes in electrical current as the molecule passes through. The single native DNA molecule long read sequencing allows for superior reading through long repeat regions and maintains strand methylation status compared to traditional next-generation sequencing. These long reads are essential for detecting structural variants include structural variants including single nucleotide variation (SNV), structural variation (SV), Insertion/Deletion (Indels), copy number variants (CNV), structural repeat regions (STR), and Methylation modification. The samples are sequenced with Oxford Nanopore Technologies PromethION P24 sequencer and the resulting reads are then subjected to quality filtering, assembly, annotation, and quality checks using Oxford Nanopore Technologies EPI2ME software and the Nanopore data analysis pipeline developed by Poochon Scientific. Genomic data generated will be 50 Gb and up to 190 Gb with the coverage up to 60X and N50 read length from 10 Kb to >100 Kb.

Applications

- **The completion of existing reference genomes**
- **Identification of undiscovered genes and their associated function**
- **Characterization of uncharted regions such as centromeres and telomeres**
- **Identification of genomic variation (SNV, SV, Indels, CNV, STR and modified bases)**

5350 Partners Court, Suite C, Frederick, MD 21703
Email: support@poochonscientific.com
Tel: 301-761-4835
www.poochonscientific.com

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Whole Genome Sequencing

by Nanopore Long Read Technology

Sample Preparation and Submission

- **Genomic DNA from any species:** ≥ 5 µg per sample, A260/A280 = ~1.8, A260/A230 = 2.0-2.2, Average size ~ 50 Kb, Concentration ≥ 200 ng/µl, Buffer: 10 mM Tris pH 8.0 or low TE (< 0.1 mM EDTA)
- **Cell pellet from any species:** ≥ 5 million cells per sample
- **Shipping:** Ship with ice packs or dry-ice

Service Description

Construction of an amplification-free long-read sequencing library using the newest ONT v14 library prep chemistry and data acquisition by ONT PromethION P24 (bases up to 190 GB; coverage up to 60X; N50 read length from 10 Kb to > 100 Kb)

Deliverables

Raw data files:

1) Fasta.gz - a compressed file of all the raw ONT sequencing reads
2) BAM (optional)

Analysis report files:

1) Reports (html) – Alignment/SNV/STR/SV/CNV
2) .fasta = polished consensus sequence of the genome
3) VCF files
4) TSV files
5) Other QC files

Turnaround Time

7-15 business days

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Workflow

WGS by Nanopore Long Read Technology

Genomic DNA Preparation

2-4 hours

Prepare high-quality genomic DNA from cells, tissues, or any biological samples. A260/A280 = ~1.8, A260/A230 = 2.0-2.2; Average size ~ 50 Kb; Conc. ≥ 200 ng/µl, Buffer: 10 mM Tris pH 8.0 buffer, or low TE (< 0.1 mM EDTA); ≥ 5 µg of DNA sample is required per sample.

Library Preparation

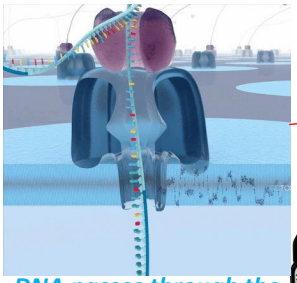
2-4 hours

Construct an amplification-free long-read sequencing library using ONT v14 library prep chemistry kits.

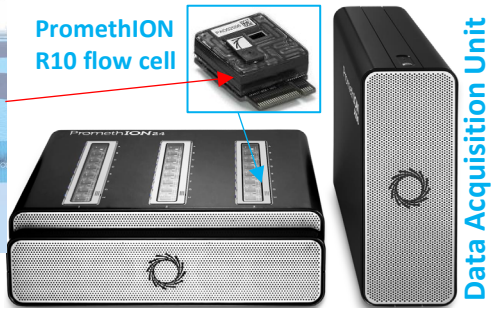
Real-time Single DNA Molecule Sequencing

24 -72 hours

Perform DNA sequencing on the PromethION P24

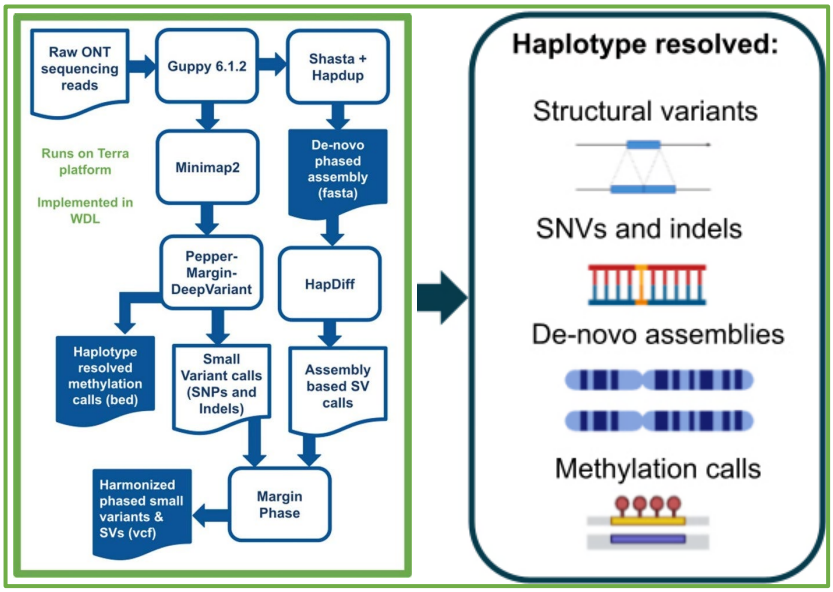


DNA passes through the nanopore at a fixed, controlled speed



Data Analysis Bioinformatics

24 hours



Data/Report Delivery

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