# Transcriptomics – Direct RNA 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 RNA eliminating PCR bias from the data. The technology feeds a single-stranded RNA molecule through a protein nanopore and measures changes in electrical current as the molecule passes through. The single native RNA molecule long read sequencing allows for superior reading through full-length and maintains strand methylation status compared to traditional next-generation sequencing. These long reads are essential for analyzing Full-Length RNA transcripts including isoforms, structural variations, Modifications, Poly-A Tail Length. 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. The data generated is from 10 Gb and up to 50 Gb with 10 M reads to 30 M reads and N50 read length ~ 2 Kb.

## Applications

- **Analyzing full-length transcripts and accurate isoform identification**
- **Analyzing full-length transcript and base modification**
- **Analyzing full-length transcript and the length of poly-A tails**
- **Rapidly identifying and characterizing RNA viruses**

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## Sample Preparation and Submission

- **Total RNA from any species:** ≥ 5 µg per sample, A260/A280 = ~2.0, A260/A230 = 2.0-2.2, Average size ~ 1.5 Kb, Concentration ≥ 200 ng/µl, Buffer: DEPC $ddH_2O$ (Water), 10 mM Tris pH 8.0, or low TE (< 0.1 mM EDTA)
- **Cell pellet samples 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 50 Gb; coverage up to 20X; N50 read length ~2.0 kb)

## Deliverables

**Raw data files:**

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

**Analysis repor files:**

1) Reports (html) – Transcriptome
2) .fasta = polished consensus sequence of the genome
3) VCF files
4) TSV files
5) Other QC files

## Turnaround Time

7-15 business days

# Workflow

## Direct RNA Sequencing

**mRNA Preparation**

*2-4 hours*

**Prepare high-quality RNA from cells, tissues, or any biological samples.** A260/A280 = ~2.0, A260/A230 = 2.0-2.2; average size ~ 1.5 Kb; concentration ≥ 200 ng/µl, Buffer: DEPC ddH$_2$O, EB, or low TE (< 0.1 mM EDTA); ≥ 5 µg of DNA sample is required per sample.

**Library Preparation**

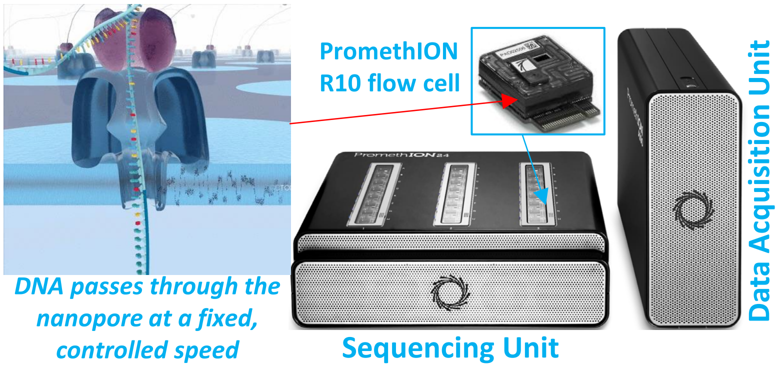
*2-4 hours*

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

**Real-time Single DNA Molecule Sequencing**

*24 -72 hours*

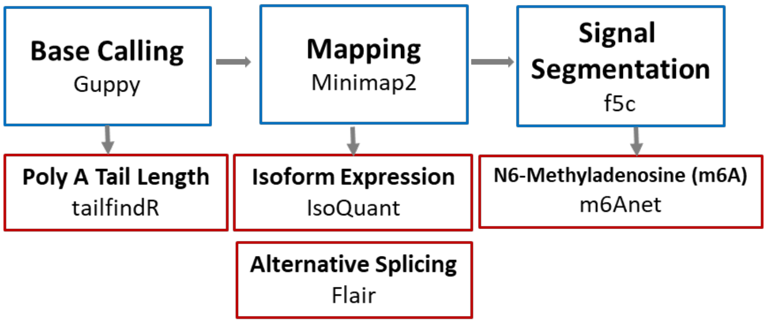
**Perform sequencing on the PromethION P24**



*DNA passes through the nanopore at a fixed, controlled speed*

**Bioinformatics Data Analysis**

*24 hours*



**Base Calling**
Guppy

**Mapping**
Minimap2

**Signal Segmentation**
f5c

**Poly A Tail Length**
tailfindR

**Isoform Expression**
IsoQuant

**Alternative Splicing**
Flair

**N6-Methyladenosine (m6A)**
m6Anet

**Data/Report Delivery**

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