Novogene

Single Cell Long Read Transcriptome

Traditional single cell assays have relied on short-read sequencing, which loses valuable information about transcript isoforms relevant to health, development, and disease. Combining long-read sequencing with single cell assays enables the unambiguous identification of alternative splicing at single cell resolution.

Our Key Features & Advantages

Overcome Shortcomings in Single Cell Short Read

- Isoform-level gene expression of RNA transcripts.
- Analyze gene expression and genomic variation at the single-cell level.
- Analyze different isoform, alternative splicing, fusion genes, etc.

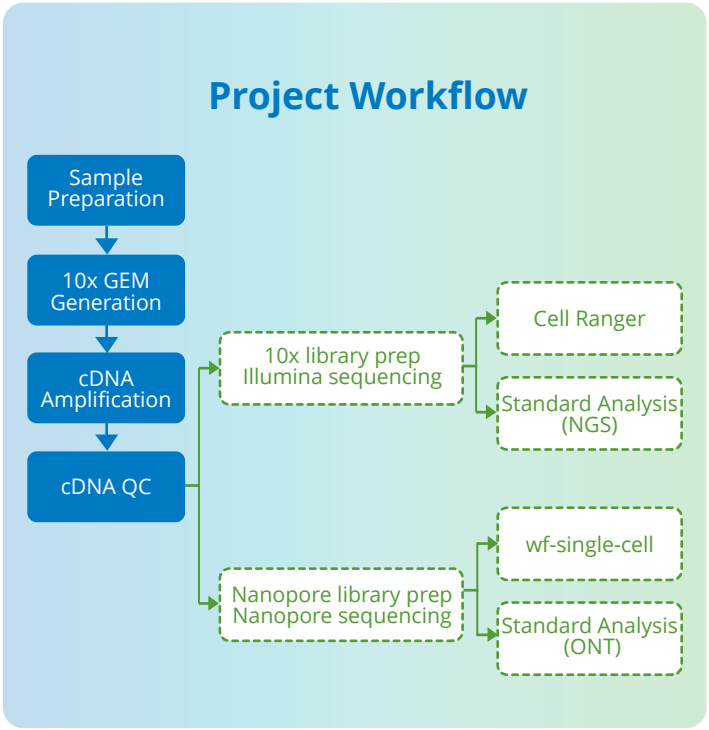
End to End Solution

- We provide single cell short read and long read transcriptome service using both Illumina and Nanopore sequencers.

Multiple Analysis Pipelines

- We have data QC pipeline and standard analysis pipeline for both short read single cell and long read single cell data.

Project Workflow



Sample Requirements and Data Suggestion

Sample Type	Sample Amount	Concentration	Others	Data Suggestion
Single cell suspension*	≥ 1,000,000	—	Cell viability: ≥ 80% Cell size: < 30 μm	Illumina: 100 - 120 Gb
cDNA derived from 10x GEM	≥ 50 ng	≥ 2 ng/μL	Peak size: 1-1.8 Kb	1 PromethION Cell

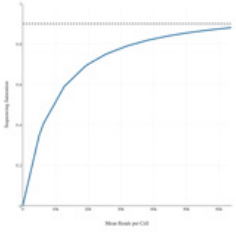
*Please refer to Novogene single cell product flyer and website for detailed sample requirement.

Analysis Content

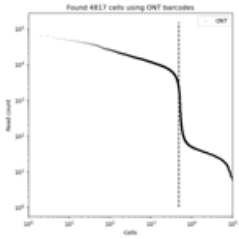
Cell Ranger (10x single cell 3' gene expression)	wf-single-cell (Nanopore single cell long read transcriptome)
1. Demultiplex BCL files from a sequencer into FASTQs 2. Summary metrics (sequencing quality, number of cells detected, the mean reads per cell, and the median genes detected per cell et al.) 3. Alignment of reads to genome 4. Gene expression quantification 5. Clustering analysis 6. Differentially expression analysis between clusters 7. Visualization	1. Data QC 2. Identify the cell barcode and UMI sequences present in Nanopore sequencing reads 3. Summary metrics (read quality, number of cells, genes and transcripts identified within each sample, median genes per cell, and sequence saturation) 4. UMAP projections

Standard Analysis (10x single cell 3' gene expression)	Standard Analysis (Nanopore single cell long read transcriptome)
1. Demultiplex BCL files from a sequencer into FASTQs 2. Alignment, UMI counting, metrics summary 3. Identification of highly variable gene (HVGs) 4. Cell Subpopulation Identification: Principal component analysis (PCA) Identify clusters of cells Dimensionality reduction and Visualization 5. Marker gene detection (Differentially expression analysis between clusters) 6. GO/KEGG/Reactome enrichment Functional annotation of transcription factor Protein-Protein interaction network analysis	1. Data QC 2. Mapping and quantification 3. Dimensionality reduction, clustering, and differential analysis Base on gene Base on transcripts 4. GO/KEGG/Reactome enrichment analysis 5. Alternative splicing

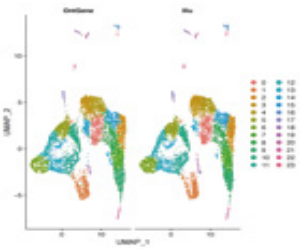
Demo Analysis Results



Sequencing Saturation (Cell Ranger)



Gene Saturation (wf-single-cell)



UMAP plots show high consistency of the cell annotation grouping results in both short reads and long reads sequencing data.

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