MEDICAL COLLEGE OF WISCONSIN

Linda T. and John A. Mellowes Center for Genomic Sciences and Precision Medicine

Single Cell RNA Sequencing (scRNA-Seq)

Test Description

Single Cell Sequencing (scRNA-Seq) provides scientists with an understanding of how individual cells respond to changes in conditions, treatments, gene variants, and a wide variety of other study designs. By sequencing as many at 10,000 individual cells per sample, rare cell types may be identified, and differential expression of individual cells can be separated from a heterogeneous population.

Sample Types and Requirements

Viable Single Cell Suspension: 5,000 to 100,000 cells (concentration range of 500 to 2,000 cells/uL preferred) with greater than 80% viability, consultation with the lab is requested

Isolated Nuclei: Consultation with the lab is requested

Tissue: Consultation with the lab is requested

Recommended Sequencing Depth

20,000 to 50,000 unique reads per cell for up to 10,000 cells (paired end, 28bp x 91bp)

Submission Requirements

Sample Intake Form and iLabs request. Single cell projects are time sensitive, thus specific handoff arrangements with the lab are required

Turnaround Time

3-4 weeks for fastq files only

2-3 additional weeks for bioinformatics analysis

Deliverables

cDNA and library quality control (PDFs)

scRNA-seq report (html with linked documents) with Cell Ranger output files and gene to cell assignment, Seurat cell cluster visualization, and unsupervised differential expression

Cell Ranger Output, fastq.gz files

All annotated NGS data files will be delivered via Mellowes Center portal

Test Methodology

Isolated cells are counted and viability checked on the Luna FL cell counter. Optimal cell numbers are aliquoted and prepared using the 10x Genomics Chromium and Next GEM Single Cell Reagent Kit. The quality and quantity of the cDNA library is checked by fragment analysis and qPCR respectively. Libraries are then pooled, and distribution confirmed on the Illumina MiSeq before sequencing is completed on the Illumina NovaSeq.

Bioinformatic Core Analysis

scRNAseq report includes:

- Quality control and sequencing metrics (per cell barcodes, mitochondrial gene content)
- Cell Ranger Analysis and Summary output
- Seurat analysis with cell cluster visualization (UMAP or t-SNE), cell counts per cluster, and PCA for high variance genes with heatmaps
- Differential expression (DE) analysis to identify the top biomarkers associated with each UMAP cluster of cells
- DE analysis in pairwise manner between experimental conditions for each cluster and violin plots of top genes with largest DE across any cluster
- Additional analysis can include supervised cluster identity, DE of specified genes of interest, pseudo-time analysis and more

Contact & Submission

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