State of the Core: NUSeq in 2024

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The Mission of NUSeq

NUSeq is committed to providing project support on:

- Next-gen sequencing,
- other genomics technologies,
- with integrated bioinformatics and data mining

Wet Lab: Tarry 2-770 / Morton 4-625; Bioinformatics: Rubloff 11-144

NUSeq Team
Most Requested NGS Applications

- Bulk RNA-seq (from standard to ultra-low RNA input)
- Single cell sequencing
- Spatial transcriptomics
- ChIP-seq
- ATAC-seq
- DNA methylation sequencing
- Whole exome sequencing
- Whole genome sequencing
Other Genomics Services

- Illumina bead arrays: for genotyping and epigenomics (DNA methylation) studies
- NanoString nCounter: direct detection of up to 800 gene expression targets
- Digital droplet PCR and real-time qPCR: target DNA and RNA analysis, and validation of NGS results
- Cell line authentication
- DNA/RNA extraction
- Sanger sequencing (via ACGT)
Bioinformatics Services

• Support analysis of all data generated in the Core
• You can also bring your own data to us for analysis
NUSeq Fiscal Year 2023 Core Utilization Numbers
(2022-09-01 to 2023-08-31)

• 762 Direct Users (Service Requesters)
• 357 PIs/Groups, 288 NU + 69 External
• 82% of services completed for NU users, 18% for external users
• $3.7M: Users spent on NUSeq services
2024 Sequencing Updates
Short Read Sequencing

• 2024 is a major transition year
• Focus will be on latest technologies
• Services on some of the old sequencers are being discontinued
  - NovaSeq 6000
  - HiSeq 4000
Short Read Sequencing
Latest technologies to focus on

**Illumina NovaSeq X Plus**
- 1+ and 3+ billion reads per lane
- $1,250 - $2,800 per lane
- Top data throughput
- Most cost effective

**Element AVITI**
- 250 - 500 million reads per lane
- 2-week turnaround
- Low to intermediate throughput
- Quick turnaround time

**Complete Genomics DNBSEQ-G400**
- 100 - 450 million reads per lane
- 2-week turnaround
- Low to intermediate throughput
- More flexibility
How to Select the Right Sequencer

• How many reads are needed
  - Over 1 billion reads: suggest NovaSeq X Plus (replaces NovaSeq 6000)
  - 250-500 million reads: Element AVITI (replaces HiSeq 4000)
  - As low as 100 million reads: Complete Genomics G400

• Turnaround time: AVITI and G400 have shorter TAT

• Cost efficiency:
  - All better than previous sequencers
  - Running 25B flow cell on NovaSeq X Plus is most cost effective for high volumes of data (e.g., high volumes of single cell or whole genome sequencing)
Long Read Sequencing

Oxford Nanopore MinION & PromethION (running)

PacBio Revio (upcoming soon this year)
When to Use Long Read Sequencers

- Genome assembly
- Structural variant detection
- Full length transcript sequencing for splicing isoform detection
- Epigenomics: direct calling of DNA/RNA modifications without derivatization
- Any applications that need long reads
Single Cell Sequencing Updates
Single Cell Sequencing

- 10x Chromium X based single cell sequencing is still the most requested
- New technologies are also available

Parse analyzes 1 million cells in a single sample (running)

Bioskryb scDNA+RNA (coming soon)
Single Cell Sequencing

Fix Sample & Nucleosome Depletion → In situ Tagmentation → Bisulfite Conversion → Library Generation → Sequencing & Analysis

Single cell methylation sequencing (Scale Bioscience, coming soon)
Spatial Transcriptomics Updates
Spatial Transcriptomics

Latest Acquisition

- Subcellular resolution: 200 nm optical resolution
- Fresh Frozen (FF) and FFPE samples compatible
- Section thickness: 10 um for FF, 5 um for FFPE
- Sample imageable area: 10.45 x 22.45 mm
- High sensitivity achieved using hybridization-based in situ sequencing
- High specificity from the use of barcoded padlock probes
- Targeted analysis of hundreds to thousands of genes, using predesigned human/mouse brain panels among others
- Allows IF and H&E staining, or Visium whole transcriptome analysis on the same tissue section

10x Xenium In Situ System (running, certified service provider)
Spatial Transcriptomics

Existing Platforms

• Spatial whole transcriptome analysis
• Resolution: 50 um (2 um with upcoming Visium HD)
• Fresh Frozen (FF) and FFPE samples compatible, FFPE preferred
• Tissue capture area: 6.5 x 6.5 mm (x4, v1), 11 x 11 mm (x2, v2)
• Protein panel and IF staining doable on the same tissue section

Visium System with CytAssist (running)

Visium HD to be released by 10x this year
Spatial Transcriptomics

Other Available Platforms

Molecular Cartography (partnership, running)

Stereo-Seq (coming soon)
Use Case of Single Cell Sequencing Coupled with Latest Spatial Transcriptomics
Single-Cell Resolution Spatial Analysis of Multiple Sclerosis Development

I. EAE MOG35–55/PTx/CFA
   peak EAE n=4
   D30 PSO n=3

II. reference scRNA-seq
   glia
   OLG
   neurons
   immune and vascular cells
   gene panel curation
   computational and manual selection
   padlock probe library targeting 239 markers

III. *
   library preparation mRNA
   sequencing
   de-novo cell typing

*Acquired by 10x and developed into Xenium

Spatial Organization of Various Cell Types

- Astro: astrocytes
- MiGL: microglia
- MOL: mature oligodendrocytes
- OPC/COP: oligodendrocyte precursor cells and differentiation-committed precursors


Interactive dataset at: https://tissuemaps.scilifelab.se/web/2023_spinal_brain.html
Cell Compartmentalization Analysis


Relative cellular composition of the different compartments.
Connections between Cell Types from Spatial Neighborhood Analysis

Summarization of 2024 NUSeq Updates

- Short read sequencing focuses on latest technologies
- PacBio Revio HiFi long read sequencing will greatly strengthen LRS
- More single cell sequencing options are available, not only on RNA, but also DNA and methylation sequencing
- More spatial transcriptomics platforms established, with 10x Xenium In Situ System being the latest offering
- Integrated single cell sequencing and spatial characterization creates unprecedented opportunities
Thank you

Questions?