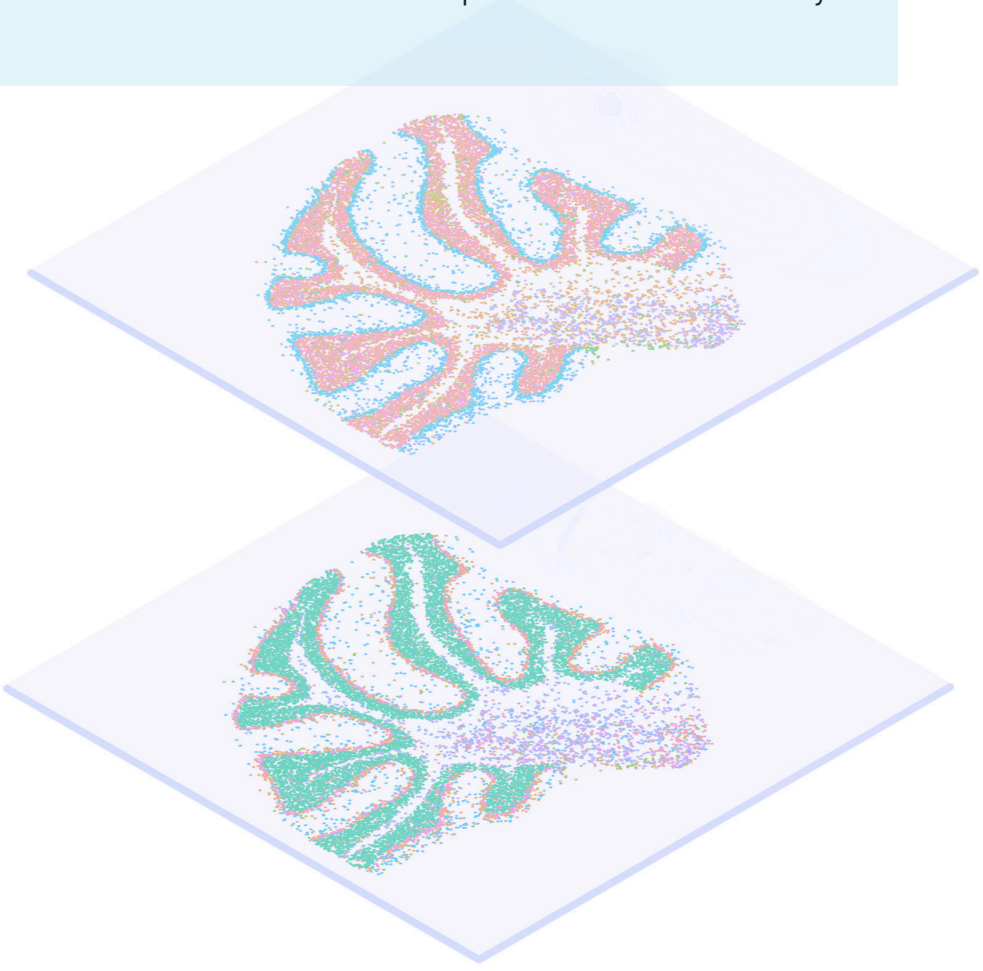
Single Cell and Spatial Transcriptomics at Strand

Background

Strand Life Sciences has been actively engaged in a diverse range of projects, including single-cell RNA sequencing (scRNA-seq), TCR sequencing (TCR-seq), spatial transcriptomics, and CITE-seq analysis. We have collaborated with multiple academic and pharma/biotech clients on projects ranging from understanding immune responses to developing novel therapeutic approaches across various therapeutic areas, including oncology, immunology, and neuroscience. This document provides an overview of key projects.



Case Studies

1. scRNA/TCR-seq Data Analysis of Uveitis Patients

Client: Uveitis and Ocular Immunology at LV Prasad Eye Institute, Hyderabad, India

Need: Comprehensive analysis of scRNA and TCR-seq data from eye vitreous fluids of 20 Uveitis patients to uncover relevant insights and support publication efforts.

Strand's service:

- Performed scRNA and TCR-seq data analysis for 40 samples (20 gene expression and 20 TCR-seq).
- Generated Cell Ranger statistics, pre-QC, and post-QC reports for all 40 samples.
- Performed scRNA seq analysis using R-based Seurat package and cell type annotation using ScType.
- Measured T-cell repertoire diversity, assessed clonality, and characterized cells into different categories (hyper/large/medium/small/non-expanded).
- Used clonal visualization to determine the total number of unique clones and examined the relative distribution of clones by abundance.
- Facilitated sample comparison based on clonal type.
- Provided publication-specific support, including joint authorship contributions.

Tools used:
Cell Ranger, Seurat, DESeq2, ggplot2, GSEA, ClusterProfiler, scRepertoire

Impact:
The manuscript is under submission. The client has extended Strand's services to analyze additional samples

2. CITE-seq Pipeline Development for CAR-T Cell Therapy

Client: A manufacturing and biotech company based out of San Diego, USA

Need: Develop a pipeline for CITE-seq data analysis to advance CAR-T cell therapy research and development.

Strand's service:

- Designed and implemented a CITE-seq data analysis pipeline tailored for CAR-T cell therapy.
- Delivered a robust and scalable pipeline for complete analysis from FASTQ to final report generation.
- Performed RNA and protein immunophenotyping characterization

Technologies used:
Fluent Biosciences & Singleton.

Impact:
Successful delivery resulted in extension of the collaboration to further enhance the pipeline.

3. Curio-seq (Slideseq2) Data Analysis of Mouse Brain after Specific miRNA Treatment

Client: Mt. Sinai School of Medicine, New York, USA

Need: Analyze spatial transcriptomics data to assess the impact of specific miRNA treatment on mouse brain tissue.

Strand's service:

- Performed complete data analysis using Curio-seq (SlideSeq2)
- Developed an integrated Slide-seq spatial transcriptomics analysis, covering quality control, data filtration, normalization, regression, clustering, visualization, and cell type annotation, ensuring the advancement of only high-quality data for further analysis.
- Identified gene expression patterns in both the Cerebellum and PFC, KO vs WT samples for comparative analysis (Fig. 1a and 1b).
- Performed comparisons of selected gene expression from ROIs, clustering on individual ROIs, and detecting subtle expression changes through log fold analysis (Fig. 2).
- Generated publication-specific images to support the client's manuscript preparation.

Tools used:
R, Seurat, RCTD, Slingshot, SPARK, scVelo, Monocle, Dtwclust

Impact:
Provided critical insights into the spatial gene expression changes induced by miRNA treatment. This contribution supported publication efforts, with the Strand team listed as co-authors.

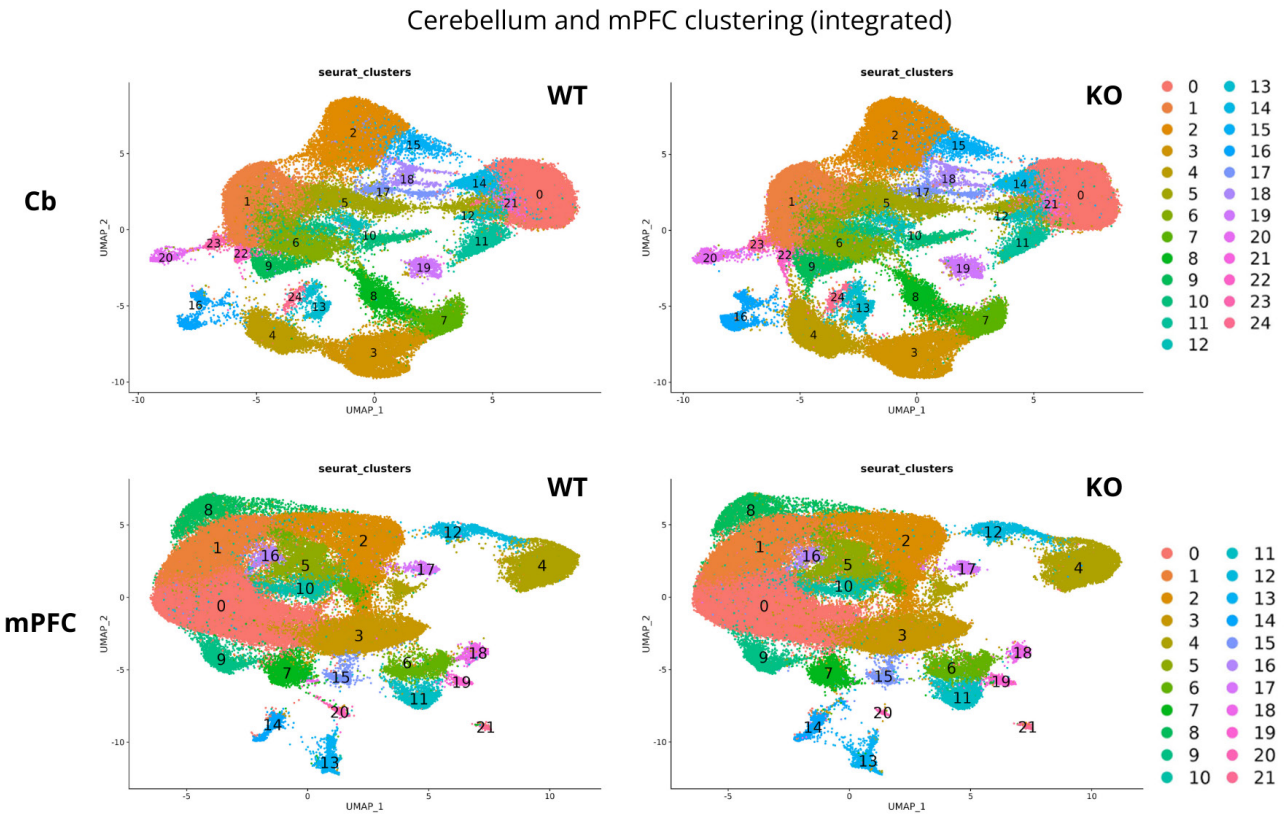


Fig. 1a: UMAP plots generated for Cb and mPFC show that tissue-wise gene expression is comparable

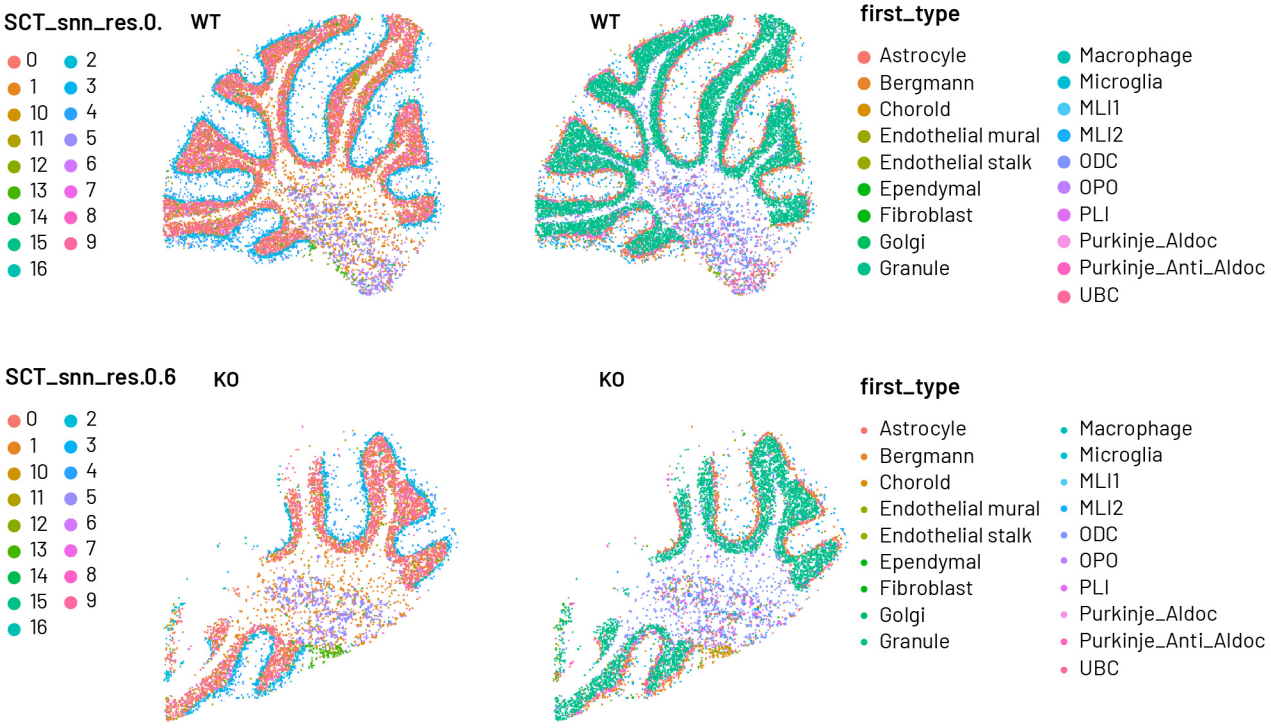


Fig. 1b: Final images of WT and KO mPFC on tissue slides with annotated cell types, confirming high-quality integration and the presence of diverse cell types across the samples

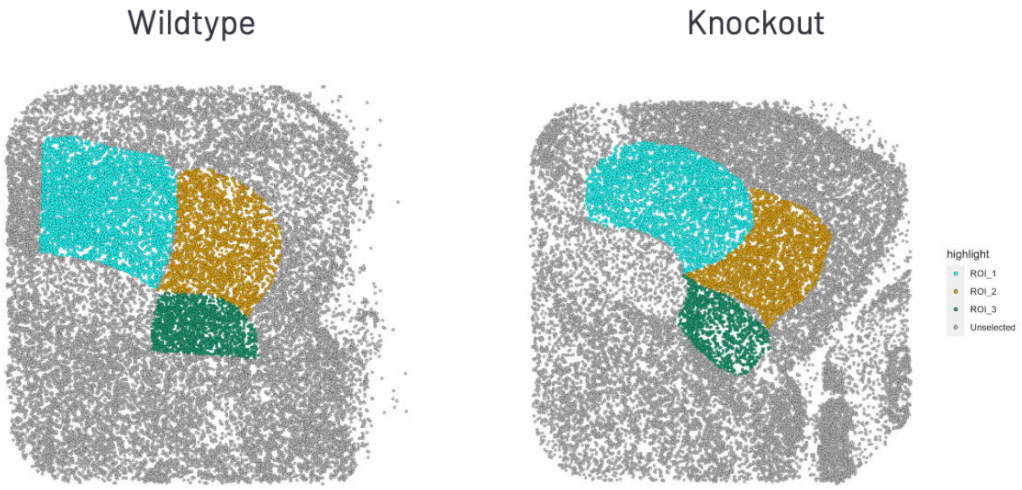


Fig. 2: The team developed a novel ROI analysis pipeline for Slide-seq data, enabling precise gene expression analysis in specific brain regions, such as the deep cerebellar nuclei (DCN). This approach allows for detailed comparisons between experimental groups (WT vs. KO), assessment of specific marker effects in targeted regions, and clustering within ROIs.

04. Nanostring CosMx Data Analysis of Human Lung Samples

Client: University of California, Irvine, USA/ A CLIA laboratory at Irvine, USA

Need: Evaluate the immune response differences in response to viral infections comparing young and aged lungs using spatial transcriptomics data generated from the Nanostring-CosMx platform.

Strand's service:

- Performed comprehensive data analysis of 8 tissue sections across two slides.
- Enhanced cell segmentation using Cellpose3, addressing issues like missing cells and false positives.
- Provided condition-specific comparative analyses, enhancing the ability to address the client's specific research questions (young vs aged).
- Conducted cell type annotation by integrating advanced annotation algorithms like ScType, SingleR, and Seurat label transfer classifying cell types; while UMAP plots and spatial images were used for precise localization on tissue slides.
- Delivered actionable insights and supported result interpretation for publication purposes.

Tools used:
Cellpose3, R, Python, ScType, SingleR, Seurat, ImageJ

Impact:
Enhanced Cell Segmentation - the Strand pipeline captures a significantly higher number of actual cells, improving analysis accuracy and providing deeper insights into cellular characteristics. The pipeline successfully captured the majority of cells positive for transcript expression and filtered out cells with fewer than 10 transcripts per cell, hence enhancing data accuracy.

05. Digital Spatial Profiling of Breast Cancer

Client: A top-20 pharma company, Indianapolis, USA

Need: Establish Visium for FFPE analysis using ER-positive breast cancer samples to advance spatial transcriptomics capabilities in clinical research.

Strand's service:

- Conducted parallel profiling of 16 samples in collaboration with the pharma company, utilizing Strand's expertise in spatial transcriptomics.
- Assessed performance on FFPE samples with varying quality
- Performed quality control by evaluating mitochondrial/ribosomal content discrepancies to ensure data quality. Provided automated threshold calculations to identify outliers, analyzed statistical parameters (mean, median, quartiles), and calculated the number of cells filtered at each cutoff, culminating in a CSV file to aid optimal threshold selection.
- Integrated batch correction algorithms (CCA, Harmony, and Liger) into the pipeline to address batch effects, ensuring differences reflected true biological variation rather than technical artifacts, thereby enhancing pipeline robustness.
- Provided manual and automated methods for cell-type annotation, including ScType, SingleR, Seurat label transfer, and RCTD (Fig. 3a).
- Provided visualization of cell annotations on tissue slides.
- Assessed correlation between IHC of breast cancer markers and gene expression.
- Compared the pathological annotations with those derived from bioinformatics analysis (Fig. 3b).
- Delivered high-quality data and insights critical for poster presentation and publication.

Tools used:
Seurat, R, SingleR, CCA, Harmony, Liger, DESeq2, RCTD, SctType,

Impact:
The findings were presented at the AGBT conference and a whitepaper was published. The project showcased Strand's ability to deliver on cutting-edge spatial transcriptomics, using the 10X platform. This project resulted in Strand being certified as a 10X certified provider for spatial transcriptomics in India.

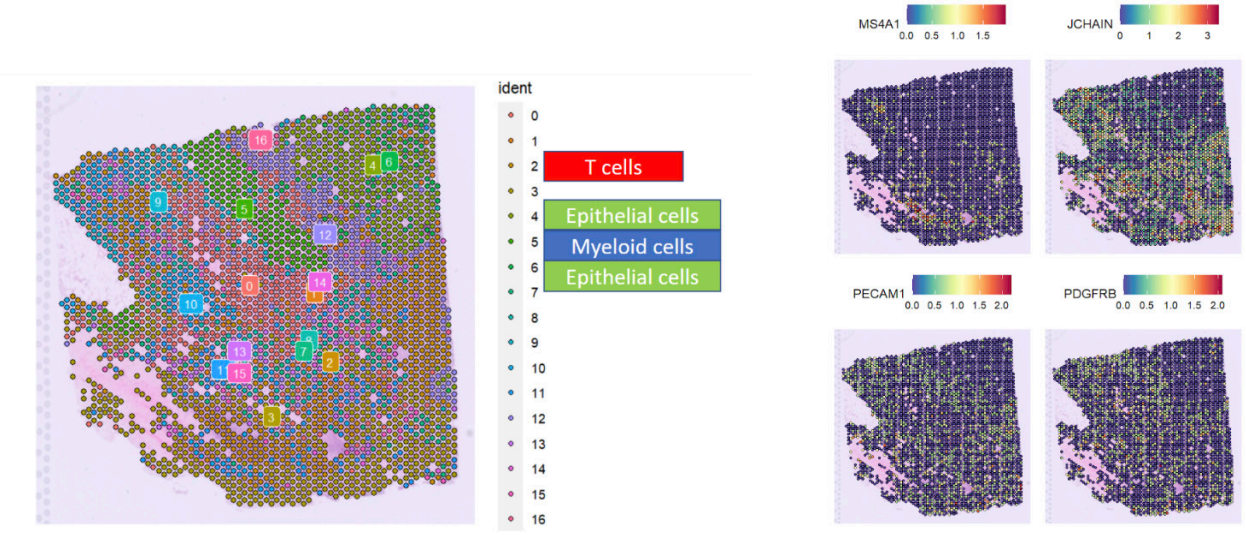


Fig. 3a: Markers for B cells (MS4A1), Plasmablasts (JCHAIN), Endothelial cells (PECAM1) and Mesenchymal cells (fibroblasts/perivascular-like cells; PDGFRB)

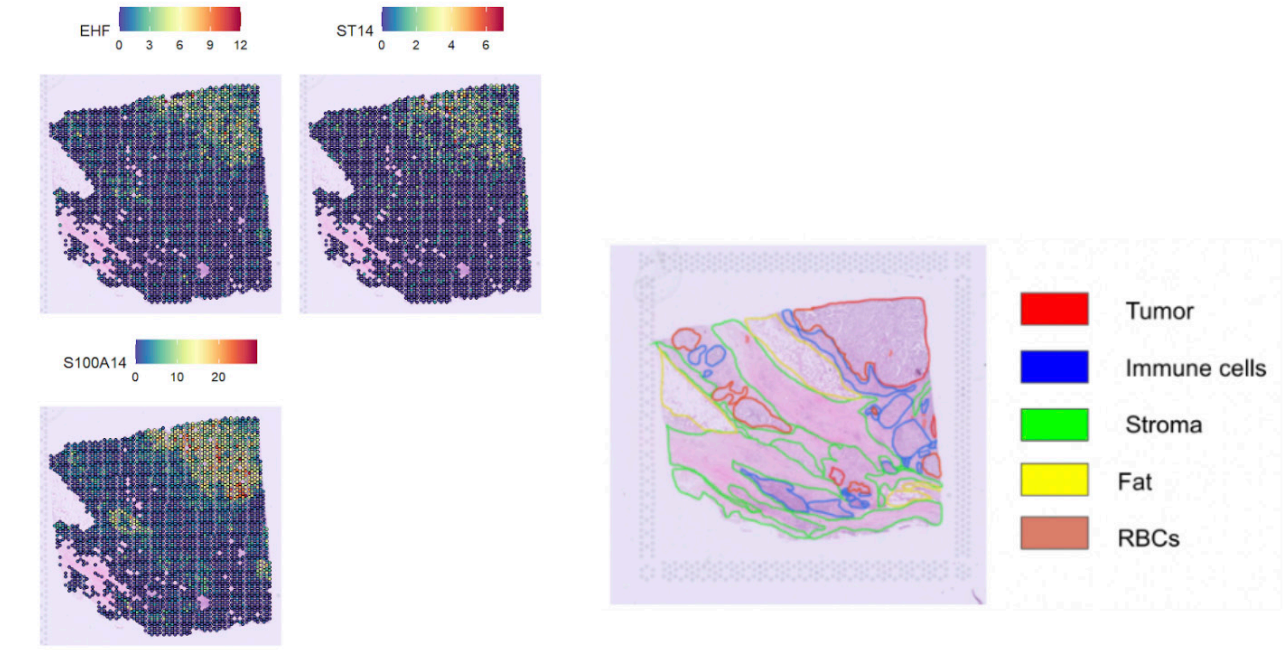


Fig. 3b: Combined analysis of breast cancer samples using Seurat 4.2

06. Visium CytAssist Profiling of TNBC TMA

Client: Rajiv Gandhi Centre for Biotechnology, Trivandrum, India

Need: Spatial transcriptomics profiling on triple-negative breast cancer (TNBC) tissue microarray (TMA) blocks, each containing 9 samples with data generated using Visium CytAssist, to study tumor biology at high resolution.

Strand's service:

- Providing expertise in Visium CytAssist technology to analyze TNBC TMA blocks
- Generating metrics such as total UMI counts, mitochondrial gene content, and the number of detected genes to filter low-quality data.
- Performing Spatial Gene Expression Profiling to visualize the expression level of genes and Identification of genes that are differentially expressed in specific tissue regions, such as tumor, stromal, or immune compartments.
- Performing comparisons of selected gene expression from ROIs, clustering on individual ROIs, and detecting subtle expression changes through log fold analysis.
- To deliver spatial transcriptomics insights essential for ongoing research.

Tools used:

Seurat, R, GSEA, Enrichr, ClusterProfiler, Giotto,

Impact:

The project is currently in progress.

07. Visium Profiling of Breast Cancer (Manual Method)

Client: Saha Institute of Nuclear Physics, Kolkata, India

Need: Visium spatial transcriptomics profiling on mouse tissue samples.

Strand's service:

- Providing expertise in manual Visium profiling to analyze breast cancer samples from mouse tissues
- Providing end-to-end pipelines to handle sequencing-based spatial transcriptomics data
- Facilitating automated methods to calculate the threshold for outliers to remove poor quality data.
- Performing batch effect correction using CCA/Harmony/Liger for combined sample analysis.
- Studying marker genes expression pattern.
- Providing detailed classification and mapping of cell types to enhance the understanding of the cellular responses to various conditions and facilitate the exploration of cellular interactions and functions within the tissue context.
- To deliver spatial transcriptomics insights critical for ongoing research.

Tools used:

10x Genomics Space Ranger, Seurat, SpatialDE, Giotto, Harmony, ScType, SingleR

Impact:

The project is currently in progress.

STRAND BACKGROUND

Strand Life Sciences, founded in 2000, provides the following services to the major instrument, diagnostics, reagent, and pharma companies:

1. Develop, validate, and interpret data from omics assays
2. Develop, test, and deploy platforms for the interpretation of omics data
3. Design and validate NGS panels
4. Analyze and interpret omics data

Highlights:

- Strand has a wide range of experience in design/development/validation and running of small to large genetic panels in the diagnostics and translational research space at its CAPand NABL (ISO 15189:2012) accredited laboratory.
- We provide assay interpretation and NGS data interpretation services to CLIA germline, somatic, and rare disease testing labs
- We developed Strand NGS, desktop software to analyze NGS data. Strand NGS is used by ≈100 researchers in the US and includes modules for RNA-Seq and single-cell RNA-Seq, from FASTQ to counts and FASTQ to cell clusters respectively
- We work with a large pharma and diagnostics company (revenue: >=$20bn) to develop bioinformatics pipelines and software to accompany a sequencing instrument
- We work with a leading liquid biopsy company (funding: >$1bn) to develop platforms for the analysis of data from their clinical trials

Over 20 Years of Bioinformatics Excellence in Advanced Genomic Solutions

The Strand bioinformatics team has a proven track record of success having completed several diverse projects for major US healthcare companies and in-house initiatives:

Genomics Biotech & Large Pharma - Eg., Custom pipelines

- Develop, test and and implement
- pipelines to analyze CRISPR assays Implementation of pipelines in Nextflow & Snakemake

Strand India R& D Omics data pipelines

- Fragmentomics analysis
- Spatial transcriptomics data analysis
- Single cell transcriptomics data analysis

Top-20 pharma - Eg., Data Analysis

- RNA-Seq data analysis to infer mechanisms causing difference in cytokine production observed in samples of interest

Strand India Clin.Dx NGS data pipelines

- 7 different bioinformatics pipelines supporting secondary and tertiary germline/somatic NGS panel data interpretation

Client References

- References from Illumina, Agilent, Roche, and other Strand clients are available on request.
- Sample testimonial: "We were very impressed with the quality of work and timeliness; you're definitely our go-to for bioinformatics consulting." - Oncology Lead, Illumina

> We were very impressed with the quality of work and timeliness; you're definitely our go-to for bioinformatics consulting
>
> - Director, Bioinformatics, Illumina

> We were immensely impressed by Strand's ability to rapidly recruit a substantially sized clinical cohort of cancer patients, and to design and run a complex liquid biopsy panel on samples drawn from the cohort, all in roughly a year's time.
>
> - Dr. Nishant Agarwal
> Chief of Otolaryngology-Head and Neck surgery and director of Head and Neck Surgical Oncology, University of Chicago.

> We have been using the StrandOmics pipeline to analyze and generate a report for our clinical cancer panels for over three years now. i would highly recommend using it to analyze data generated from clinical cancer NGS panels and the outputted clinical report provided after analysis.
>
> - Senior Scientist/ Medical laboratory director for NY State, Prim Bio Research Institiute

24+ YEARS OF EXPERIENCE

80,000+ Genetic Tests Reported

500+ Projects Executed for Genomics Majors Globally

Presence in **20+** Countries

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