



ABRF Corporate Partner Presentation



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Single Cell Sequencing: From Intake to Analysis

A Practical Guide for Core Facilities and the Researchers They Serve

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Presenter:

Brian Davis

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Summary

Single-Cell Transcriptomics Overview

Brian provided a comprehensive overview of single cell sequencing workflow, covering experimental design considerations, sample preparation best practices, platform selection, and post-sequencing data quality metrics. He emphasized the critical role of genomics cores in supporting researchers through the complex single cell process, particularly for new users. The presentation included detailed discussions on choosing between fresh cells versus fixed nuclei, experimental replicates, quality control metrics, and data filtering approaches. The session concluded with a brief Q&A period where participants asked about the relative usage of single cells versus nuclei in research, to which Brian responded that approximately 60% of users work with cells while 40% use nuclei, depending on experimental requirements.

Brian explained the advantages over bulk RNA-seq using the smoothie analogy to illustrate how single-cell analysis can identify individual cell types and rare populations that might be masked in bulk experiments. He discussed technological advancements in single-cell platforms, highlighting how microwells, microfluidics, pico wells, and nano droplets have enabled profiling of increasing numbers of cells from hundreds to potentially millions. Brian emphasized the importance of working with genomics cores for experimental design guidance and QC metrics throughout the single-cell process, particularly for researchers new to the field.

Single Cell Sample Considerations

Brian discussed key considerations when deciding between working with fixed or fresh samples for single cell experiments. He explained that fixed samples offer advantages including removing time constraints, enabling batch processing, and reducing batch effects, while fresh samples may be more suitable for immediate processing. Brian also covered important factors in sample preparation including the decision between capturing cells or nuclei, the need for technical and biological replicates, and maintaining high viability and quality throughout the process. He emphasized the importance of consulting with core facilities and reviewing recent publications in the field to ensure successful single cell experiments.

Single-Cell Workflow Best Practices

Brian discussed best practices for single-cell workflow, emphasizing the importance of moving samples into fixation quickly and using dual methods for assessing cell morphology and viability, including trypan blue and fluorescent dyes. He highlighted the RIN score as a crucial indicator of RNA integrity, particularly for bio banked tissues, and recommended testing tissue quality before proceeding with single-cell experiments. Brian also stressed the importance of accurate cell counting, whether manual or automated, and discussed the role of genomics core facilities in generating essential quality control metrics during library construction.

Core Facilities and Sequencing Protocols

Brian discussed the importance of utilizing core facilities for library preparation and sequencing, emphasizing the need to provide specific details about sample delivery, tube types, concentrations, and assay requirements. He highlighted the critical importance of correctly conveying library structure and read information to sequencing providers to avoid losing barcodes and reducing experiment resolution. Brian also explained key sequencing metrics, including barcode rank plots, gene transcript levels, sequencing saturation, and valid barcode fractions, using examples to demonstrate high-quality data outcomes.

Single Cell RNA Sequencing Overview

Brian presented on single cell RNA sequencing, covering sample quality metrics, data processing workflows, and quality control considerations. He explained the importance of working with core facilities during sample preparation and highlighted different analysis tools including Parse's Trailmaker tool for users with limited bioinformatics experience. When asked about single cell versus single nuclei applications, Brian indicated that about 60% of users choose single cell approaches while 40% opt for single nuclei, with nuclei being particularly useful for fixed tissue samples.