ABRF Research Agenda

Key Studies Planned for 2023

September 2022

The **Association of Biomolecular Resource Facilities (ABRF)** Executive Board is pleased to announce the approval of several innovative studies to be conducted by ABRF Research Groups in 2023, to address ongoing questions in biomolecular research techniques and methods.

ABRF Research Groups bring together leaders in biomolecular sciences and techniques to attempt to answer questions or address challenges. Over the course of 12-18 months, these Groups conduct original research that often results in new findings, publications, and presentations at ABRF and other scientific meetings.

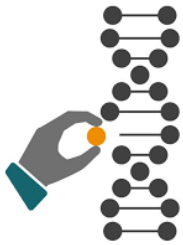
> *"ABRF Research Groups are often referred to as the 'heart and soul' of ABRF as these groups take deep dives into scientific questions associated with best practices in processing samples and generating and analyzing data in a scientific core. These new RG projects are outstanding examples of the impactful research that ABRF Research Groups perform to enable core scientists to make informed decisions that improve the services they provide. Many thanks to ABRF corporate partners for their continued support to ABRF Research Groups."*

Kevin Knudtson
ABRF President

ABRF Research Groups benefit from generous support from corporate partners to contribute to these important projects, through in-kind donations of kits, reagents, and other research materials, or through direct financial support for shipping samples or select travel for Group members to collaborate in person. ABRF also provides modest funding to support shipping costs and other related study expenses.

For 2023, the ABRF research agenda includes:

Validation of New Materials on the Market for CRISPR-mediated Knock-ins



Genome Editing Research Group (GERG)

This study will compare the efficiency of 'protein tag' knock-ins. This will be accomplished by comparing the materials using client projects across multiple laboratories. Each participating lab will use materials generated as part of a project requested by a core facility client, for a custom edited cell line. For example, one of the participating laboratories in this study may use this method to test targeting a HALO tag fusion to the C-terminus of a gene of interest. On-target integration can be assessed by PCR analysis across the homology arm junctions.

Multi-site Assessment of Methods for Cell Preservation Upstream of Single Cell RNA Sequencing

DNA Sequencing Research Group (DSRG)

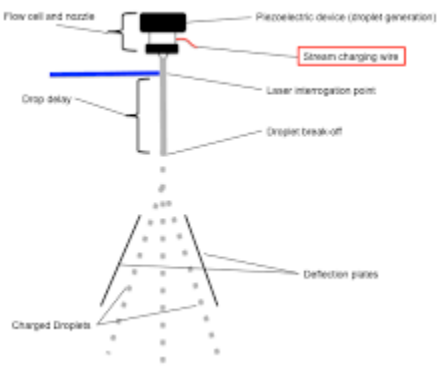
The objectives of this study will be to prepare a total human leukocyte sample for use in downstream workflows. This will include:

- Prepare a total human leukocyte sample for use in downstream workflows, then perform flow cytometry to generate reference data for cell type identification and relative abundance within the human leukocyte sample
- Process single cells using the 10x Genomics v3.1 workflow prior to preparing the cells for cell preservation

- Prepare total human leukocytes for long-term storage following protocols outlined by 10x Genomics (Fixed RNA Profiling), Parse Biosciences (Evercode WT) and Honeycomb Bio (HIVE)
- Distribute frozen, preserved samples to sites for QC and NGS library preparation using assigned workflow
- QC final libraries and ship to a single site for sequencing
- Partner with ABRF's Genomics Bioinformatics Research Group (GBIRG) for downstream data analysis, interpretation, and manuscript preparation

"New methods and assay improvements for single cell preservation and storage are changing the way research is conducted and our thorough investigation into the performance of each will provide a valuable resource to assist scientists and core facility managers to determine the most appropriate single cell preservation workflow given their sample collection logistics and laboratory infrastructure constraints."

Jessica Podnar
University of Texas
Chair, ABRF DNA Sequencing Research Group



Testing the Accuracy of Drop Delay in Sorters with Automated Drop Delay Calibration

Flow Cytometry Research Group (FCRG)

This study seeks to:

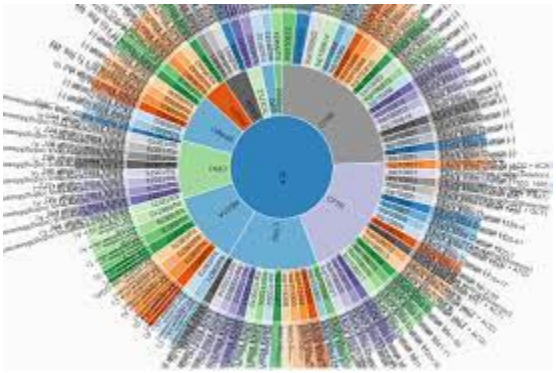
- develop a protocol to test beads of various diameters on sorters with automated drop delay calibration compared to sorters with manual drop delay calibration;
- using beads of specific diameters, sort beads to test the accuracy of sorting and sort yield when using automated drop delay calibration compared to manual calibration;
- determine the accuracy of sorting and sort yield as it relates to the make/model of sorter

"Successful cell sorting is a fine balance between sample preparation and cytometer operation. As a critical part of the process, determining the purity of the sort by calibration of the time it takes for the cell of interest to get from detection by the laser to the drop, otherwise known as drop delay, is fundamental. The data collected in this study will help serve as a guide for sort operators to determine whether drop delay is set up accurately, and whether a sorter is set to the best conditions for sort accuracy, purity, and recovery."

Christiane Hassel
Indiana University-Bloomington

Jane Srivastava
Gladstone Industries
Co- Chairs, Flow Cytometry Research Group

Bioinformatics Assessment of Fixed/Frozen Single-Cell RNA-Seq Data across Three Platforms



Genomics Bioinformatics Research Group (GBIRG/DSRG Collaboration)

"This study will inform providers of single-cell RNA-Seq services about the performance of new fresh, frozen, and fixed cell partitioning approaches in the context of a well characterized biological material. As part of this study, our research group will also deploy a shared computational environment that will facilitate collaboration between members at different institutions. This project is a collaboration between DSRG and GBIRG and we are excited for the opportunity to foster interactions with our counterparts in the DSRG."

Shaun Polson
University of Delaware
Chair, GBIRG

The aims of this study are to:

- Prepare count matrices from 10XGenomics, Honeycomb and Parse
- Sequence data using commercially supported software. Investigate the possibility of using open-source software (Salmon/Alevin) to prepare similar count matrices from FASTQ file.
- Attempt to establish equivalence between the methods through comparison of QC metrics, global expression profiles, and comparisons with publicly available bulk RNA-Seq datasets.
- Down sample FASTQ files and reprocess the data using either commercial or open-source software.
- Subset cell sets to assess performance of various methods with decreased coverage and population sizes.
- Configure and deploy a shared R-instance that can be accessed and utilized by members of both the DSRG and GBIRG so that all code development and analytical activities can occur in a consistent, shared environment.
- Perform standard quality control analyses and make comparisons of QC metrics such as number of features detected, count of UMIs/cell and per cent mitochondrial reads. This processing will include ambient RNA adjustment and doublet detection and doublet rates will be compared between platforms.
- Execute data integration and batch correction to produce a single Seurat object containing all cells from each site and platform.
- Perform unsupervised clustering analyses to identify the total number of robust clusters and enumerate the site and platform-specific contribution to each cluster.
- Perform cell type classification of the resulting clusters to test the hypothesis that some cell types (granulocytes) are captured more effectively using different technologies.
- Perform differential gene expression analysis to identify cell-type markers and make cross-platform comparisons of these results at both the gene and gene set levels.

ABRF's 2023 Research Group Studies are made possible through the generous support of these ABRF Corporate Partners:

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ABRF welcomes additional Corporate partners to support this valuable work. With additional resources, each study has the potential to be expanded to include greater data collection and analysis.

Visit the ABRF web site for more information on ABRF Research Groups. To support this valuable work, contact ABRF Executive Director Ken Schoppmann to discuss how your group can be involved.