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Poster Abstract Book

Combining single cell chromatin landscape and gene expression with spatial transcriptomics to characterize Alzheimer's Disease progression in CRND8 APP mice

Michelle Bell, Stephan Prokop, Stephen Williams, Luigi Alvarado, Kristy Dillon, Valeria Giangarra, Juan Romero, Michael Gibbons, Todd Golde, Yona Levites, Kelly Miller, Alaina Puleo

Abstract:

Objectives: Alzheimer’s Disease (AD) currently impacts 6 million Americans who are living with the disease and 1 in 3 seniors die with a diagnosis of AD or dementia. Given the epidemiological state and impact of AD, a better understanding of the biology of the disease is needed to develop effective preventative and curative therapies. Here, we demonstrate the benefits of combining both single cell and spatial information in TgCRND8 APP transgenic mice to further understand amyloid deposition and its inflammatory signature.

Methods: Using the Chromium Single Cell Multiome ATAC + Gene Expression and Visium Spatial Gene Expression with FFPE assays, the open chromatin landscape, and gene expression profiles of Tg APP-overexpressing and wild-type mice brains from 2 to 20 months old mice were evaluated. In addition, Aβ production and Aβ-associated neuroinflammation across several anatomical regions of the brain were analyzed and correlated with regulatory programs identified based on multiomic data.

Results: Results showed multi-cellular gene co-expression networks at both the single nucleus and spatial levels as well as distinct open chromatin sites during the course of amyloid deposition. These findings were noteworthy in brain regions containing Aβ plaques accompanied by a neuro-immune response from distinct cell populations as a function of amyloid deposition, e.g. C4b in oligodendrocytes within fiber tracts.

Conclusions: Together, the data showcases the utility of combining open chromatin and gene expression analysis at the single cell level with spatial transcriptomics of FFPE tissues to fully characterize cellular networks and responses in the time-sensitive context of AD.

Efficient Sample Preparation Enables Extraction, Isolation, and Purification of Total Nucleic Acids from FFPE Tissue Samples

Deb Bhattacharyya

Formalin Fixed Paraffin Embedded (FFPE) tissues are extremely valuable in modern research as it can be used to study disease progression and development, which in turn helps development and optimization of therapeutic pathways. Results obtained from samples also help to determine prognosis and in the development of therapy.

This study showcases a comprehensive portfolio of kits powered by Covaris' Adaptive Focused Acoustics® (AFA®) Technology for extraction of nucleic acids from FFPE tissue samples. These truXTRAC FFPE series of extraction and purification kits include DNA- or RNA-only as well total nucleic acid (sequential RNA and DNA from the same sample) extraction, coupled with column or magnetic bead-based purification.

The truXTRAC FFPE Total NA Auto 96 workflow supports any scalability requirements, a growing demand for most organizations working with hundreds to thousands of samples. The kit comprises 96 individual 0.5 mL Matrix tubes with automated bar-code reader-access (with automated capping/decapping) can be processed in parallel facilitated by a "Shuttle" adaptor to transfer all 96 tubes to the Covaris R230 (on-deck) Focused-ultrasonicator, the heat-blocks, automated centrifuge, capper/decapper and the magnet stations. The workflow was also improved with an initial deparaffinization step using a novel non-toxic reagent to eliminate possible problems with automated pipetting steps during supernatant removal. Subsequent AFA-treatments ensure that any residual wax is actively removed guaranteeing higher yields as well as high quality nucleic acids. The modification also allowed to both simplify and speed up the magnetic bead-based purification workflow without jeopardizing yield and quality of RNA and DNA fractions. truXTRAC FFPE Total NA Auto 96 workflow offers robust, reliable, reproducible workflows for every laboratory working with FFPE samples and enable them to achieve confident data for every sample, every time.

Vanderbilt University Qualitative Research Core

Kemberlee Bonnet and David Schlundt

The VU-QRC offers services to investigators in three six areas: 1) Formative research for the planning, development, tailoring, or revision of diabetes and obesity-related interventions; 2) Mixed methods evaluation of intervention programs; 3) Empirical development and refinement of measurement tools; 4) Evaluation of Smartphone and Web-apps intended for patient or provider use; 5) development and use of direct observation data collection tools; and 6) use of quantitative approaches that require qualitative interpretation. Qualitative research methods constitute a heterogeneous toolkit of frameworks, strategies, and techniques for answering questions using data sources that are not easily quantified. The VU-QRC explicitly employs an iterative inductive/deductive approach to qualitative coding and analysis. Deductive (theory to fact) and inductive (fact to theory) approaches are used iteratively to develop coding systems and to organize coded data into conceptual frameworks. The deductive phase requires that we explicitly identify the theoretical approach that is guiding our work such as social cognitive theory, the biopsychosocial framework, and the social ecological framework. While theory guides our understanding of larger categories and relationships among constructs and categories, the inductive phase pulls details from the coded transcripts and is used to identify themes and to create a deeper understanding of the theoretical constructs as they relate to the research problem.

Expanding Services at the University of Tennessee Genomics Core through Teaching

Veronica Brown

The Genomics Core at the University of Tennessee, Knoxville, offers a course through the Microbiology Department to graduate students and upper level undergraduates, giving students the opportunity to get hands-on experience with high-throughput sequencing in a low stress, supervised environment. Students prepare their own samples for 16S amplicon sequencing on the Illumina MiSeq, from DNA extraction, through library prep and final pooling and QC. Students are able to watch as samples are loaded onto the sequencing instrument, removing the “Black Box” feeling of handing their samples off to someone else. We have utilized multiple pipelines for quick analysis of results, including Qiagen’s CLC, DADA2 in R, and apps in Illumina’s BaseSpace. The UT Genomics Core has recently added an Illumina NovaSeq, and, while we are not currently using this higher-throughput instrument for sequencing in the class, students are exposed to the differences between sequencing instruments and get to see the differences in the instruments and flow cells. Many students report that this is the most useful class they take and continue to utilize the facility in their later research, both in their projects while at UT and after they graduate.

Low-input ultraplexed RNA-seq library kits with integrated ribosomal RNA removal

Sujash Chatterjee, Samuel Rulli

Low-input RNA-seq library preparation has several challenges. Low-input samples often make mRNA enrichment and ribosomal RNA (rRNA) removal steps difficult due to the potential loss of RNA before the cDNA synthesis steps. In addition, multiple enzymatic and bead cleanup steps after cDNA synthesis can result in a loss of diversity during each individual step. Also, for some applications where a high number of samples need to be processed, workflow inefficiencies often prevent the ability to run over 384 samples simultaneously or achieve the desired volume of NGS library preps required to conduct a small molecule or CRISPR screen.

To address these bottlenecks, the QIAGEN team has developed a new RNA library preparation kit that uses an integrated rRNA removal method with sample-specific barcodes to enable cDNA pooling. This library kit uses the same workflow regardless of RNA quality to generate either 3' RNA-seq or whole transcriptome libraries. This enables researchers to optimize their workflow based on sample number, read budgets and sequencing platform.

We have successfully developed a customizable rRNA removal kit with off-the-shelf options for human, mouse, rat and related species, pan-bacteria, yeast, fish, plants, fly and worms. This allows the workflow to be ubiquitously used with most model research organisms, starting with 500 pg to 100 ng of total RNA or the equivalent amount of enriched mRNA. Researchers can combine rRNA removal kits based on their samples. For example, our epidemiology kits allow the study of both bacteria and human gene expression from the same sample. The results from this RNA library kit show over 97% of stranded RNA without the use of actinomycin-D or dUTP, with a very low amount of rRNA contamination. The versatility of the RNA library method allows researchers to run both 3' RNA-seq and whole transcriptome libraries simultaneously. cDNA pooling of up to 24 samples enables over 18,000 samples to be ultraplexed into each

The Center for Innovative Technology: A Full-Service Collaborative Research Core for Global Untargeted and Targeted Metabolomics

Simona Codreanu

The Center for Innovative Technology offers collaborators an extensive lineup of state-of-the-art mass spectrometry instrumentation for a variety of metabolomic analyses. The CIT, in conjunction with the McLean laboratory, is designated as a Waters Center of Innovation (Waters Corporation) and recognized as an Agilent Thought Leader Laboratory (Agilent Technologies). Our team has over 40 years of combined experience in mass spectrometry, data analysis, and informatics pipelines. The CIT provides investigators comprehensive support for untargeted metabolomic analyses, offering a number of routine and advanced services depending on individual research goals. Method development efforts and user-defined analyses are also available for targeted biomolecule measurements and metabolite validation. The complex array of metabolites within a fluid, cell, or tissue can be measured and steady state or temporal changes can be determined for context-dependent global metabolomics analyses. Metabolite annotations are assigned based on high mass accuracy measurements, retention time, isotope distributions, tandem MS/MS spectra, and comparisons with in-house reference spectral libraries. Results can reveal unique biochemical fingerprints of cellular processes specific to a sample group. These types of datasets can be exploited as a discovery-based approach for generating novel hypotheses or used for a better understanding of physiological processes mediated by genetic or environmental perturbations.

Human and Mouse Cell Line Authentication Testing - Essential Quality Control Testing

Erin Hall, George Maha

In the early 1950s, the “Chang Liver” cell line was established from the biopsy of a patient during an exploratory laparotomy, and later, in 1962, deposited at the ATCC specimen biorepository for other researchers to use as a “normal liver” in vitro model. In 1967, Stanley Gartler presented evidence that Chang Liver, among other cell lines, displayed isoforms that were identical to the infamous HeLa cells (established in 1951). Today, Chang Liver is sold by ATCC; however, it is clearly labeled, “HeLa [Chang Liver]” after short tandem repeat (STR) profiling definitively confirmed that it was misidentified HeLa cells. Unfortunately, despite knowing about the misidentification, Chang Liver is still used today as a model of normal liver cells. STR profiling was also later used to help settle the controversy involved in the case of the shared donor of two commonly used human cell lines called MDA-MB-435 and M14. STR profiling helped to prove that MDA-MB-435 cells, originally thought to be derived from a breast cancer patient, were cross-contaminated after establishment by M14, a melanoma cell line. With authentication testing of early samples, it is possible to resolve the origins of problematic cell lines that are widely used in research studies. STR profiling is an established, standardized method to confirm that human and mouse cell lines are not misidentified or cross-contaminated. Misidentification and cross-contamination is estimated to affect 18-36% of all human cell lines used in research and up to 20-40% of peer reviewed publications that use human cell lines. Currently, there are more than 500 cell lines known to be misidentified or cross-contaminated.

Next Generation Glycan Microarray Enabled by Next Generation Sequencing

Yi lasanajak, Xuezheng Song

Interactions of glycans with proteins, cells and microorganisms play important roles in cell-cell adhesion and host-pathogen interaction. We developed the Next Generation Glycan Microarray (NGGM) based on artificial DNA-coding of glycan structures. In this novel approach, a glycan library is presented as a mixture of glycans and glycoconjugates, each of which is coded with a unique oligonucleotide sequence (code). The glycan mixture is interrogated by a glycan binding protein (GBP), which binds its corresponding glycan ligand(s) along with the linked DNA codes in solution. After the GBP is separated from unbound coded glycans, the DNA sequences that identify individual bound glycans are quantitatively sequenced (decoded) by next generation sequencing (NGS) technology. Copied numbers of the DNA codes represent relative binding specificities of corresponding glycan structures to GBPs. The feasibility of this system was demonstrated by using common lectins and a small library containing six DNA-coded glycans. NGGM Libraries containing 48 DNA-coded defined glycans and a shotgun library of 96 DNA-coded pig kidney glycan fractions were constructed. The glycan-binding specificities of biotinylated lectins and antibodies using NGGM compared favorably with the solid phase glycan microarray. Furthermore, shotgun array in NGGM format identified glycan binding specificity of bacterial adhesins, which is technically challenging in solid phase glycan microarray format. Compared to the solid phase glycan microarray, NGGM have advantages of high throughput, expanded dynamic range readout, and compatible assay with intact cells.

Creating a SIGnature program to support NIH S10 instrumentation grants

Amy Martinez, Jenny Schafer ,Susan Meyn ,Jessie Pirtle, Susannah Imhoff

The NIH S10 shared instrumentation grant programs provide cores an exceptional opportunity to acquire leading-edge scientific equipment. However, the unique administrative requirements can feel overwhelming for grant PIs and institutions new to S10s. In this presentation, we share an overview of Vanderbilt University Medical Center's S10 program, which has supported 21 applications in the last five years and currently has nine awards under active reporting. We outline our program's administrative structure, lessons learned, and best practices to help shared resource colleagues feel confident pursuing S10 awards. We'll also explore how we partner with Vanderbilt University to align S10 processes across our integrated network of shared resources.

Meharry core facility support of COVID-19 testing lab during a pandemic

Franklin Nouvet, Jeffrey Leegon, Jasmine Marshall-Anderson, Derek Wilus, Shanell Higgins, Atanu Khatua

OBJECTIVE: In the Summer of 2020, Meharry Medical College (MMC; Nashville, TN), along with seven other HBCUs, were approached by Thermo Fisher (TF) and the Gates Foundation (GF) to set up a COVID-19 PCR testing lab on their respective campuses (The Just Project). The aim was to partner in providing access to surveillance testing free of charge during a global pandemic. METHODS: A hub-spoke model was used to provide COVID-19 PCR testing. Hub schools established full testing labs and spoke schools collected samples and sent them to the hub for testing. MMC served as a hub for two spoke schools, Fisk University (TN) and Central State University (OH). TF paid for sample collection kits and their shipment, equipment leases, testing reagents, and licensing contracts for the SARS-CoV-2 clinical diagnostics EUA (TaqPath), the LIS, and EHR systems needed for CLIA certification. The GF funded hub school staff and other equipment. Meharry Medical College created an on-campus, CLIA certified Molecular Diagnostics Laboratory (MDL) for processing nasal swab samples. The efficiency with which the MDL was established was due to the direct support of the Meharry core facility (CRISALIS), part of our NIH-funded Research Centers in Minority Institutions (RCMI) grant. The Meharry RCMI provided program management personnel, clinical informatics expertise, and biostatistician support for data analysis. RESULTS AND CONCLUSION: By utilizing CRISALIS core lab expertise, multiple HBCU’s surveillance needs were met during the COVID-19 pandemic. CRISALIS staff led the operational logistics of testing and provided data to campus response teams. PCR results were sent via patient portal to the student or staff member, to school administrators via weekly reports, and to the state health agency over HL7 messaging. Since January 2021, more than 42,000 samples were efficiently processed (average result time of 10 hours) preventing major outbreaks on campuses and assisted data-driven policy.

Spatial Immunophenotyping using the PhenoCycler Platform at the Vanderbilt-Ingram Cancer Center Immunophenotyping Shared Resource

Jamye O'Neal, Kimberly Dahlman, Elizabeth Huddleston

Over the past several years, there has been an explosion in knowledge of host-tumor interactions and the mechanisms by which cancer cells evade host immune-surveillance, leading to incredibly successful anti-cancer therapies. Vanderbilt investigators have a critical need for high-dimensional in situ imaging analysis at cellular and subcellular levels for studying host-tumor interactions and response and resistance to immunotherapies.

The Immunophenotyping Shared Resource (IPSR) recently acquired a PhenoCycler system including the PhenoCycler fluidics instrument (Akoya Biosciences), acquisition and processing computers, and an immunofluorescence microscope (Keyence) [1S10OD030338-01]. This system allows for comprehensive, spatially-resolved, multiplexed biomarker analysis on FFPE or frozen tissue and generation of highly multiplexed panels that can detect up to 50 antigens. This highly multiplexed assay is achieved by conjugating barcoded oligonucleotides to antibodies followed by hybridization with a dye-labeled reporter for highly specific dectection and efficient melting/rehybridization cycles. When tumor tissue is limiting, it is necessary to multiplex more antigens and rapidly identify novel biomarkers per single tissue section. The IPSR successfully generated a 14-antibody panel to elucidate host-tumor interactions in head and neck squamous cell carcinoma and is in the process of generating PhenoCycler panels for other tissueand tumor types.

The IPSR has a rich history of exceptional collaborative work studying host-tumor interactions and has successful hands-on experience using the PhenoCycler System on tumor tissue. Over the past twelve years, the IPSR has collaborated on over 300 laboratory projects that has been recognized by over 40 publications. The IPSR is co-led by established faculty members with a wealth of experience in performing highly complex immunological assays, which is critical to the successful implementation of the PhenoCycler System. Dr. Jeff Rathmell, IPSR Scientific Director, and Dr. Kim Dahlman, IPSR Managing Director, along with experienced IPSR research staff provide guidance, assistance, and management of the PhenoCycler System services.

Normalizing UDI Library Construction for Sensitive Genomic Applications

Renata Santos

For a wide range of genomic applications, the ability to sensitively discriminate sequencing reads between different samples is a critical requirement to reduce the effects of noise and index contamination in NGS data. Generally, the method of unique dual-indexing addresses this by adding two sample-specific sequence indices that are unique to each sample. Most available methods that employ UDIs do so by incorporating indexing in the library amplification stage, prior to purification and pooling of libraries for multiplexed sequencing. This represents a high workflow burden because individual libraries have to be carried separately through library construction, amplification, purification, and QC adding significantly to handling time and cost.

Here, we describe a novel approach for UDI library construction that permits pooling of samples immediately after dual-indexed transposase tagging, allowing samples to be purified, amplified, and prepped for sequencing as normalized UDI-tagged multiplexed pools. This new method utilizes a novel decoy-based normalization technology during indexed tn5 transposition that generates uniform quantities of library molecules across a 10-fold range of input DNA from 5 to 50 nanograms, a working range of DNA input amount that is suitable for many common genomic workflows and applications. The 10 bp UDIs were designed to be both greater than two errors and two indels away from any other index in the set and were rigorously screened for performance across multiple sequencing chemistries.

We demonstrate the suitability of this new library construction method on a range of sample types and compare the indexing, normalization, and workflow performance to other commonly used UDI library methods. We anticipate that the method will have wide applicability to NGS workflows that have significant multiplexing requirements that also require the sensitivity and performance benefits of unique dual-indexing.

A complete, automated single-cell RNA-seq system for enabling biomarker discovery

Kazu Tori, Eric Pu, Yanli Liu, Yue Yen, Sally Zhang, Ying Bao, Lyndon Liu, Necip Mehmet, Bryan Bell, and Andrew Farmer

Takara Bio USA, Inc., 2560 Orchard Pkwy, San Jose, CA 95131

Many RNA transcripts undergo post-transcriptional modification such as alternative splicing, with tumor-specific isoforms showing clear diagnostic value as biomarkers. Full-length single-cell mRNA sequencing has contributed to the discovery of these rare biological events; however, automated, high-throughput workflows are desired. We developed the SMART-Seq® Pro kit, which employs full-length SMART-Seq chemistry on the ICELL8® cx Single-Cell System and Cogent™ NGS bioinformatics tools, for use as an end-to-end, automated solution for biomarker discovery. We compared the gene detection ability of the SMART-Seq Pro kit against the Smart-seq2 (SS2) homebrew method and assessed the ability of the SMART-Seq Pro kit to correctly identify clinically relevant splice variants of the PTPRC gene. We showed that the SMART-Seq Pro kit detects more genes than SS2, highlighting the kit's ability to reveal rare biological events. The SMART-Seq Pro kit also successfully identified PTPRC isoforms of interest, unlike 3' end counting methods. These data illustrate how the SMART-Seq Pro kit for the ICELL8 cx Single-Cell System can characterize relevant isoforms and, together with Cogent NGS analysis tools, offer an efficient way to scale up studies for biomarker investigation.

BioVU: An Administrative Hub Optimizing Institutional Resource Utility

Kathryn Trogden, Estefania Gibson, Olivia Joseph, Alicia Ferguson, Leigh Ann Saucier, Jasmine Torain, Celestial Jones-Paris

BioVU is a de-identified biorepository at Vanderbilt University Medical Center (VUMC) which facilitates investigators from basic scientists to clinicians using human data in research, a process that is typically costly and difficult to navigate with significant and nuanced regulation. The biorepository is disease-agnostic and contains ~280,000 banked samples and their associated medical record data that are stringently de-identified and are readily available for research. BioVU shoulders much of the regulatory burden to allow for more cost-effective research than can be accomplished with an identified, recruited study. To achieve this, BioVU serves as the administrative hub for multiple other cores and institutional bodies within VUMC. This allows BioVU to help investigators navigate through all stages a study, including forming a concept, project design, implementation, execution, and data utilization. BioVU has established and maintained operational relationships with sister core leadership and uses a variety of approaches for cross-team information management and communication, including various tools and regular updates. Establishing a solid network and point person as well as a mutual understanding of primary responsibilities for each institutional body has been key to the ability of investigators to successfully leverage the resource and navigate the intricacies of using human data in research. As a longstanding program, BioVU has experience with adapting to changes in technology, regulatory guidance and direct institutional support. Regular re-evaluation and adjustments to the relationships with other institutional bodies shared through open communication helps keep everything functioning efficiently through changes. In conclusion, BioVU's function as an administrative hub allows for the use of human data for all investigators regardless of career stage by reducing the overall burden and facilitating navigation through the necessary regulatory steps.

A closed loop feature detection platform for automated neonate cardio-respiratory measurements and data analysis

Christopher Ward, Mariana Garcia Acosta, Dipak Patel ,Brandon Ruiz ,Eunice Aissi, Russell Ray

Background:

Sudden Infant Death Syndrome is thought to partly result from unseen brain abnormalities affecting cardio-respiratory function, but for which no clear genetic or environmental mechanisms are known. Mouse genetic and exposure models present an opportunity to uncover molecular and environmental mechanisms. However, measuring cardio-respiratory function in neonate mice is expensive, difficult, and inefficient. One major challenge is the time needed to carry out such measurements. The neonate autoresuscitation assay, consisting of repeated anoxic exposures followed by recovery, requires the full attention of an observer for the multi-hour duration of a single-subject assay, limiting throughput. To address this, we developed a closed-loop feature detection platform for automated neonate cardio-respiratory measurements.

Methods:

Our platform design consists of:

1) a pneumotachograph face-mask for precise respiratory measurements.

2) a micro-controller automated bell-housing gas switching system for rapid induction of respiratory challenges.

3) a micro-computer-based data-acquisition system with real-time feature detection and outputs for controlling gas exposure.

4) a data analysis suite that assists with recordkeeping and provides a facile method to extract key outcome measures.

Results:

Gas challenges are administered via the rotating bell housing system. A python program detects waveform features in real-time, such as apnea and bradycardia. Upon apnea detection, the system switches to a rescue gas. Resumption of normal breathing and heart rate can also be detected and incorporated into criteria for automated initiation of the next anoxic exposure trial. Data is stored for later offline automated analysis using the data analysis suite.

Conclusions:

The system offers a relatively inexpensive approach for automated high throughput neonate cardio-respiratory assessment for screening to yield important clues in developmental pathophysiology.