WORKSHOP REPORT: NIDDK HEALTHY REFERENCE TISSUE AND STANDARDS VIRTUAL WORKSHOP ON MONDAY JULY 25TH

Executive Summary

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) held a *Healthy Reference Tissue and Standards* virtual workshop on Monday July 25th, 2022. There are multiple cell and tissue atlas programs underway around the globe that are generating community resources and attempting to answer fundamental questions about human health and disease. The goal of the workshop was to identity and prioritize research opportunities related to key needs for these atlas-building efforts.

It is becoming increasingly clear that data quality and integrity must be a major focus at all stages of the research life cycle—from collection through curation, use, and dissemination. The workshop had discussions on the key needs for all atlas building efforts including: 1) Development of a suite of reference standards for multiple molecular modalities that can be easily adopted by the research community, 2) Implementing Quality Assurance/Quality Control (QA/QC) plans and ensuring consistent, precise, and accurate measures over time, and 3) Development of appropriate "healthy reference tissue" benchmarks as comparators for disease. The workshop brought together experts from multiple fields to identify state-of-the-art, fit-for-purpose standards for the variety of multi-omic assays that are being utilized by various atlas programs and stimulate discussion of the next generation of standards. Although the goals of the workshop were to address these issues through the lens of kidney precision medicine, lessons learned from other biomedical fields enhanced discussions.

In the workshop, participants highlighted several themes including the need for QA/QC to permeate the culture of all atlas programs. In programs that involve human specimens, there is a critical need to understand how pre-analytical variables impact the data generated from various molecular modalities, and to implement and QA/QC standards to improve the rigor and reproducibility of research within and across atlas programs. The workshop participants also emphasized the need to define "normal" and "healthy" tissue. As the long-term goal of many of the atlas programs is to study disease processes in detail, workshop participants thought it was essential to understand biological variability across the lifespan, sex, race, ethnicity, and geographic area, as well as heterogeneity within populations and in normal tissue. It is essential that the data generated by each atlas program must be of adequate quality, as the data may eventually inform clinical care (e.g., used in the development of clinical assays).

Workshop participants stressed that there are many persistent misconceptions about what defines "normal" or "healthy" tissue. These definitions are being debated at the pathological level and subject to context. However, it is becoming increasingly clear that there is not a single source of tissue that is the perfect, normal reference for atlas programs. We are learning that normal, healthy tissue is quite heterogenous and what is reported as "normal" can contain abnormal pathology, cells, and/or molecular signatures. To understand healthy variation more

fully, participants thought it important for atlas programs to adequately account for sources of biological variability listed above. Many speakers and participants noted that the role of context is often forgotten when obtaining human tissue and critical metadata needs to be captured, collected, and shared. For instance, many thought it was important to address the status of a patient's immune system at the time of biopsy.

One interesting area of discussion centered around altruistic or volunteer research biopsies, which were thought to be an important source for understanding normal, healthy tissue variability. Although the workshop participants noted that the ethics of obtaining altruistic biopsies are evolving, going forward, it is of the upmost importance to demonstrate that the research tissue donations will be used properly to benefit society and handled with the utmost care and subject to rigorous QA/QC. IRBs are recognizing patient autonomy in determining whether to allow non-minimal risk research. Significantly, it is becoming clear that cadaver material has substantial limitations.

Overall, the workshop focused on quality and was defined by several themes. 1) Quality must permeate the culture in atlas programs and cannot be an afterthought. QA/QC and corrective and preventative action (CAPA) plans need to be designed at the outset of every consortium. 2) Every step in the analytic process needs appropriate standards and benchmarks, especially pre-collection and pre-analytical steps, which are often underappreciated and critical for project success. 3) Assay performance should be monitored frequently and tracked over time using analyses like Levy-Jennings plots, which are instrumental in identifying quality control issues. 4) Orthogonal and independent validation is vital to verify existing data and distinguishing effects versus artifacts. 5) Research quality requirements should be built into clinical quality requirements as molecular assays may be used for making patient decisions in the future. 6) Internal standards do not need to be perfect but must have a few highly reproducible elements that can serve as positive and negative controls over time. 7) Fit-for-purpose reference materials as standards are needed to QC samples and track data longitudinally. 8) Metadata quality control challenges are often overlooked, and it is important that all quality control steps (e.g., protocols, QC results, performance against benchmarks) are shared with the research community in an accessible library.

WORKSHOP REPORT

DESIGN AND DEVELOPMENT OF THE WORKSHOP

An Organizing Committee (consisting of Drs. Carolyn Compton, Jeff Spraggins, Ben Neely, Christina Jones, Petter Bjornstad, Tony Dickherber, Tara Hiltke, and Jasmin Bavarva) was recruited to work with NIDDK staff members (Eric Brunskill, Danny Gossett, and Chris Ketchum) to construct the meeting agenda (See Appendix 2 for agenda). The meeting was structured around four broad topic areas to define the state of the field and to address key questions related to quality control standards and healthy reference tissues. Session One: Lessons learned from NIH Atlas Programs. Session Two: Understanding the Dual Role of "Healthy Tissues" as Standards and "Normal" Reference Tissue. Session Three: Developing Assay-specific Community Standards: Current State-of-the-art Standards: Lessons from the Bench. Session Four: Lightning talks: Late-breaking Innovative Ideas. Moving the Field Forward and Developing Next Generation Fit-for-purpose Standards. The speakers in each session were tasked with addressing key, topically focused questions, such as:

- *What quality control metrics and what standards should be adopted for a variety of molecular technologies?*
- *What are the sources of variability that are most troubling?*
- *What are the challenges that remain in the field going forward?*

The preliminary background presentations served to frame and focus breakout sessions that were organized around specific technologies or topics. There were six breakout discussion sessions designed to cover topics related to Pre-Analytics, Healthy Reference Tissues, Transcriptomics, Proteomics, Metabolomics and Lipidomics, and Epigenomics. Discussion leaders were assigned to each of the breakout groups and tasked with guiding workshop participants to address the following questions:

- What are the current best practices?
- What are the limitations to current best practices (pro and cons)?
- What improvements or new developments need to be made? What can be attained or achieved in one year? Five years?
- What community-wide experiments are needed to validate new technologies and standards?
- What potential sources of variability do you worry about most?

SUMMARY OF BREAKOUT SESSIONS

The breakout sessions were designed to address quality control challenges that face modern atlas programs and to identify new opportunities, standards, and reference materials that the research community can use to enhance the rigor and reproducibility of atlas datasets. Meeting participants in breakout sessions provided recommendations to place QA/QC issues at the research forefront and identified short-term and long-term goals (outlined below) as priorities for the research community.

Group 1: *Pre-analytics*

- *Pre-analytics is the unsung but all-important baseline infrastructure for science and the various atlas programs.*
- *There is a vital need to improve devices for collecting and storing biospecimens in a manner that minimizes pre-analytical changes and variability.*
- *There is a need to develop quality management systems that include minimum standards for collecting patient characteristics. Pre-analytical factors that will impact the molecular integrity and the molecular composition of the biospecimen must be monitored and documented.*
- ***Short-term:** Implement systems for detailed, standardize capture of biospecimen history and patient data.*
- ***Long-term:** Develop a seamless connection between the practice of basic, translational, and clinical research, in which the molecular integrity of specimens is safeguarded and quality increased.*

Group 2: *Healthy Reference Tissues*

- *There is no such thing as "normal," as health is a spectrum and physiology varies over the life course and with other biological variables.*
- *There is a critical need for atlas programs to include the history of the tissue (metadata) and clinical phenotyping and to build an understanding of how these data vary within the population.*
- *There is a need to develop and use standardized protocols to facilitate comparative analyses across international cohorts*
- ***Short-term:** Study different tissue sources to leverage strengths and address limitations, and better understand biological variability.*
- ***Long-term:** Make well-defined reference tissues and standards widely available and deploy across atlas programs. Possibly develop tissue engineered mimetics.*

Group 3: Transcriptomics

- *Pre-analytical variables, including tissue processing, have a significant impact on measured transcriptomes. Improved metadata capture is needed to 1) better understand the impact of specific variables and 2) address potential bias and sources of library preparation artifacts.*

- *Atlas programs should interrogate replicate biospecimens to evaluate computational pipelines and bioinformatics.*
- *The research community needs sustainable reference materials. Currently, there are limited supplies of standard materials for transcriptomics. Make artificial cells that have enough in common to be leveraged as assay standards.*
- ***Short-term:** A reference tissue should be circulated by a biorepository/biobank to multiple atlas programs to test concordance of protocols, assays, and technologies**.***
- ***Long-term:** Sample processing protocols and technologies should be developed and integrated with the aim of deriving the maximum number of assays from limited biospecimens (e.g., small mass of biopsy tissue).*

Group 4: Proteomics

- *"Proteomics" is an umbrella, under which there are diverse methods (immunoassays, imaging, mass spectrometry, apatmer-based assays, etc.) each with very different workflows and needs.*
- *Orthogonal techniques for validation and rigor are recommended but are difficult to identify.*
- *There is a need to disseminate state-of-the-art QC metrics and software. "The future is already here; it's just not very evenly distributed."*
- ***Short-term:** Atlas programs should make QC data public to aid in secondary analysis, re-use, and protocol sharing, as well as supporting development of a proteomic description of "normal."*
- ***Long-term:** Atlas programs should include the use of bio-printed, cell-based standards for spatial MS and Liquid chromatography–mass spectrometry (LC-MS) approaches, and develop scalable materials for wider sharing, which have built-in copy-number for quantification purposes.*

Group 5: Metabolomics/Lipidomics

- *Development or selection of standards needs to account for not only heterogeneity within a tissue but differences in composition between tissues or organs (e.g., tissue with low versus high lipid content).*
- *The mass spectrometry community needs to develop and identify common standard reference materials (SRM). It should develop an understanding of how metabolomics and lipidomics profiles vary across the lifespan.*
- *The research community needs concise and well-documented protocols that can be shared across atlas programs.*
- ***Short-term:** Multiple atlas programs should incorporate a common, shared SRM in a cross-atlas effort to test protocol and technology variability. Strategies are needed to address challenges of storing and analyzing large datasets, as well as to enable smaller labs to access these data and bioinformatic platforms.*
- ***Long-term:** The research community should develop more complex reference materials that mimics the physicochemical environment of different organs and can be used across several modalities.*

Group 6: Epigenomics

- *It is important to note that epigenomic technologies may be deployed in concert with other -omic technologies (e.g., scATACseq is deployed with scRNAseq to assist in cell-type identification), and the common quality control needs of both technologies should be considered.*
- *Selection of computational approaches is a source of variability in epigenetic analysis (for example, differences in peak calling algorithms). There is a need to standardize pipelines and validate results using multiple, orthogonal approaches.*
- *Several areas to improve epigenetic studies include the use of synthetic nucleosomes as quality control standards, antibody standardization, mass spectroscopy validation, and generation of an atlas of epigenetic benchmarks.*
- ***Short-term:** Common, standardized QC metrics, sequencing depth, and sequencing metrics reporting, as well as sharing data platforms, should be adopted by the scientific community and atlas programs.*
- ***Long-term:** Atlas project should add other epigenetic technologies, beyond ATAC-seq (e.g., beyond looking at chromatin accessibility), and integrate epigenetics with other molecular technologies to annotate cell-states.*

Cross-cutting Themes from Breakout Discussions

1. Close attention to pre-analytic variables is essential.
2. Quality control **must** permeate the culture in all facets of a consortium.
3. There is no such thing as "normal." As such, there is a need to study different reference tissue sources to leverage strengths and address limitations.
4. There is a need to develop novel fit-for-purpose standards that closely mimic different tissues and can be used across several modalities
5. Well-defined reference tissues and standards should be made widely available from a central source and deployed across atlas programs, and QC data should follow the tissue and be made available to the public.

Acknowledgements:

First and foremost, the NIDDK would like to thank the Organizing Committee members: Drs. Carolyn Compton, Jeff Spraggins, Ben Neely, Christina Jones, Petter Bjornstad, Tony Dickherber, Tara Hiltke, and Jasmin Bavarva. Their critical insights helped guide the process through articulation, design and chairing the different sessions during the workshop. The NIDDK is also grateful to the other discussion leaders: Drs. Sanjay Jain, Charles Wang, Chris Anderton, Michael Rauchman, and Mike Eadon. The NIDDK also thanks all workshop speakers and participants for their enthusiastic contributions to the meeting.

Appendix 1: Lightning Talk Abstracts

Improving spatial N-glycomics for human kidney using reference tissue and robust quality control

Dušan Veličković[1], Kumar Sharma[2], Theodore Alexandrov[3], Guanshi Zhang[2], Jeffrey B. Hodgin[4], Christopher R. Anderton[1]

[1]Pacific Northwest National Laboratory, Richland, WA, USA. [2]Center for Renal Precision Medicine, The University of Texas Health, San Antonio, TX, USA. [3]Structural and Computational Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany. [4]Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA.

Understanding the kidney glycome and its spatial variation is of critical importance, because glycoconjugates serve as anchoring sites for cell adhesion, extracellular matrix molecules, signaling receptors, and pathogens. Moreover, glycosylation is increasingly reported to be a key factor in many biological processes, including disease progression. Within the glycome, N-glycans are of notable importance, as they are the major class of carbohydrate modifications present in 90% of all glycoproteins. Within the last decade, spatial N-glycomics with matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) has become an increasingly popular method in clinical research, as this method offers the ability to measure aberrant and changing N-glycan profiles spatially within a tissue section with microanatomical and often single-cell resolution. Within the Kidney Precision Medicine Project, our team has built a robust spatial N-glycomics platform for biopsy tissues. A significant advantage to imaging N-glycans with MSI is that FFPE tissues can be utilized. We found that detected N-glycans, their spatial distributions, and relative signal intensities are robust and can be retrieved from sections of tissue stored for over a year. This opens an avenue to analyses of archived kidney tissue for studying the spatial N-glycome. Moreover, we developed a method for using a reference human nephrectomy tissue as a QC sample to reduce batch effects and instrument drift over time. Another benefit to using FFPE tissue is that samples tend to be better preserved, and retain morphological and cell structures more readily, in comparison to fresh/frozen preserved tissues. This allows us to more confidently identify the N-glycans that localize to specific cell types and functional tissue units. As such, we have developed the ability to quantify molecular delocalization that results from sample handling and preparation. We recently utilized this approach to systematically improve our spatial N-glycomics assay in effort to maximize N-glycan sensitivity and mass spectrometry ion image clarity (Fig. 1).[1] Lastly, as part of our efforts, we created NGlycDB, a public database of naturally occurring N-linked glycans and added it to METASPACE.[2] METASPACE is a high-performance big data infrastructure for MSI data. METASPACE helps by performing N-glycan annotation based on the accurate mass, spectral isotope pattern, spatial co-localization of isotopic peaks, and measure of spatial chaos, which is integrated into a statistically rigorous False-Discovery Rate-controlled bioinformatics framework. Thus, our experimental workflow, NGlycDB database, and the cloud software METASPACE provide an integrated solution for spatial N-glycomics.

Nephrectomy tissue as a source for high quality reference tissue

Markus Bitzer MD, Christopher O'Connor, Greg Teichert PhD, Meghan Dailey, Andrew Hlynka, Brenda Gillespie PhD, Kerby Shedden PhD.

University of Michigan, Department of Medicine: University of Michigan, Department of Statistics

Background: The kidney is a vital organ and acute and chronic kidney injury is associated with increased morbidity and mortality, decreased quality of life and increased health care costs. Furthermore, treatment options are limited and available test to detect kidney damage are very insensitive and unspecific. Therefore, novel insights into the processes that lead to kidney disease are of very high significance. Interrogation of the kidney is very challenging due to the complex structure of the kidney which includes glomeruli, tubuli, vessels and interstitial area. Each of these compartments are composed of different cell types, and exhibit distinct responses to diseases and stressors, but also interact with each other. New technologies, in particular single cell and spatial omics methods have provided novel insights into physiology and pathology of the kidney. Because the kidney structure changes not only with disease but also with age, sex and heritable factors, distinguishing "normal" or "healthy' versus abnormal or pathologic changes can be challenging and may be context dependent. For advanced omic platforms optimal tissue collection and processing are important. Therefore, percutaneous kidney biopsies in clinically healthy volunteers are

considered the gold standard. The major limitations of these types of samples include the very small amount of tissue available significantly limiting the ability to share tissue for different interrogation methods from the same person and increasing the sampling bias.

Method: Therefore, we studies kidney tissue obtained from patients undergoing nephrectomies which allows collection of much larger pieces of tissue which can be shared between many groups, used for many different interrogation techniques and allowing accurate assessment of the health of the kidney tissue. To optimize kidney tissue quality, we established a unique protocol (PRECISE cohort):
- careful patients selection [majority of kidney tissue being unaffected by the mass (>5 cm distance of the tissue collection site from the mass), undergoing standard nephrectomy surgeries (which include clamping of the vascular pole just prior to removal of the kidney), exclusion of patients with ureteral obstruction or compression of vascular pole]
- minimizing cold ischemic time (close communication with operating room staff)
- minimize tissue processing (use of a 3D-printed device to cut tissue into either slides or biopsy like cores to allow rapid saturation with preservatives)

Results: Preliminary data show that
- the samples have been successfully used for single cell and spatial transcriptomics and proteomics.
- results generated in these samples have been validated in percutaneous kidney biopsies
- clinical parameters are very insensitive in detecting pathologic changes
- larger kidney tissue sections allow capturing rare histologic phenotypes
- significant heterogeneity of pathologic changes within one sample exist and pathologic features appear to cluster together suggesting significant risk for sampling error.

Conclusion: Nephrectomy tissue is a safe way to obtain large amounts of high-quality kidney tissue which can shared for multiple omic methods allowing for cross-validation of findings. Furthermore, it allows for more accurate assessment

Quality control metrics for epigenetic interrogation technologies
Michael T. Eadon, Michael I. Rauchman

Background: Molecular atlases of the kidney have largely focused on transcriptomic signatures of kidney cell types. Mature interrogation technologies such as single cell RNAseq now have well-accepted quality control metrics which facilitate data integration across technologies and consortia. Quality control metrics for epigenetic interrogation technologies such as Methyl-sequencing, CUT & RUN, and scATACseq are needed.

Methods: In human kidney tissue, we tested reproducible quality (QC) standards for whole genome bisulfite sequencing (WGBS, N=30) of dissected glomeruli (GLOM) and tubulointerstitium (TI), bulk Cleavage Under Targets & Release Using Nuclease (CUT&RUN, N=12) with H3K27ac and H3K27me3 antibodies and single cell Multiome (combined snRNAseq and scATACseq, N=12) to integrate these technologies. Peak alignment in genomic features (promoter, exon, CpG island, etc) was determined by Fisher's exact test.

Results:
We describe the key Go and No-go criteria at the pre-analytic, experimental, and post-analytic stages of sample interrogation. QC measures are given for batch correction, PCA clustering, controls, and reproducibility for WGBS, CUT&RUN, and scATACseq in the human kidney. Go/No-Go criteria for CUT & RUN include DNA recovery as compared to an IgG control antibody and peak size distribution on bioanalyzer. Go No-go criteria for WGBS include DNA quantity and quality, mapping rates and PHRED scores. The use of a methylated and unmethylated control are employed. Technical replicates from nephrectomy controls are run and the correlation between samples is assessed to screen for batch effect. Levy-Jennings plots assist in identifying technology drift. Both CUT & RUN and WGBS are integrated with scATACseq to align peak calling between the three technologies, adding orthogonal validation and confidence to H3K27ac peaks and WGBS valleys.

Conclusions: The QC measures described may provide a robust and reproducible dataset for analysis across consortia. The orthogonal validation of epigenomic features provides an integrated view of histone modifications and DNA methylation which contribute to chromatin accessibility in the kidney.

Long-Term Metabolomics Reference Material

Goncalo J. Gouveia, PhD.; Amanda O. Shaver, PhD.; Brianna M. Garcia, PhD.; Alison M. Morse, PhD.; Erik C. Andersen, PhD.; Arthur S. Edison, PhD. and Lauren M. McIntyre, PhD.

The use of quality control samples in metabolomics ensures data quality, reproducibility, and comparability between studies, analytical platforms, and laboratories. Long-term, stable, and sustainable reference materials (RMs) are a critical component of the quality assurance/quality control (QA/QC) system; however, the limited selection of currently available matrix-matched RMs reduces their applicability for widespread use. To produce an RM in any context, for any matrix that is robust to changes over the course of time, we developed iterative batch averaging method (IBAT). To illustrate this method, we generated 11 independently grown Escherichia coli batches and made an RM over the course of 10 IBAT iterations. We measured the variance of these materials by nuclear magnetic resonance (NMR) and showed that IBAT produces a stable and sustainable RM over time. This E. coli RM was then used as a food source to produce a Caenorhabditis elegans RM for a metabolomics experiment. The metabolite extraction of this material, alongside 41 independently grown individual C. elegans samples of the same genotype, allowed us to estimate the proportion of sample variation in preanalytical steps. From the NMR data, we found that 40% of the metabolite variance is due to the metabolite extraction process and analysis and 60% is due to sample-to-sample variance. The availability of RMs in untargeted metabolomics is one of the predominant needs of the metabolomics community that reach beyond quality control practices. IBAT addresses this need by facilitating the production of biologically relevant RMs and increasing their widespread use.

A common sample for cross platform, multimodal quality control standards of spatial resolution

Melissa A Farrow, Martin Dufresne, Angela Kruse, and Jeff Spraggins

Two key quality control areas have emerged as tissue mapping projects evolve and drive at higher spatial resolutions. First, the critical question of measuring and calculating spatial resolution. Second, the ability to normalize signal intensity across samples and modalities. To address both, we have developed a sample consisting of a mixture of Caco2 cells transduced with GFP and 293T cells transduced with H2B-mCherry prepared in a gelatin solution. The resulting cell pellet can be sectioned and thaw mounted for acquisition of fluorescence signal, lipid IMS, and Nanostring GeoMx profiles. The high spatial resolution of fluorescence microscopy can be leveraged to measure and calculate the spatial resolution of downstream modalities. Additionally, the signal intensity output from the cell pellet can be utilized to normalize signal intensity across a series of experiments. By providing a common quality control sample that can be distributed and implemented across experimental workflows, we can now standardize measurements across modalities as well as samples and labs. This approach will facilitate the acquisition of reproducible and accurate multimodal, spatially targeted datasets.

Appendix 2: Agenda

July 25, 2022

10:00 a.m. – 10:10 a.m. **Welcome**
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Robert Star, M.D.,

Key Needs of Multiple National Institutes of Health (NIH) Atlas Efforts

- To develop a suite of reference standards for multiple molecular modalities that can be adopted easily by the research community to improve quality assurance/quality control (QA/QC) and ensure consistent, precise, and accurate measures over time
- To develop appropriate "healthy reference tissue" benchmarks as comparators for disease

Key Question for Workshop Participants

- What healthy reference tissue(s) and/or standard(s) should I use when I run an experiment in my laboratory next week?

10:10 a.m. – 10:50 a.m. **Session One: Lessons Learned from Atlas Programs**

10:10 a.m. – 10:30 a.m. **"Lessons Learned from Atlas Programs"**
Carolyn Compton, M.D., Ph.D., Arizona State University

10:30 a.m. – 10:50 a.m. **"Lessons Learned from HuBMAP and nPOD Programs"**
Mark Atkinson, Ph.D., University of Florida

Key Questions for Speakers

- What do we know now about pre-analytics, QA/QC, and the inclusion of standards?
- What are the challenges and current best practices for minimizing batch effects and technical variability to understand biological variability?
- How do you prepare for different end user needs?
- What potential sources of variability did you worry about most?

10:55 a.m. – 12:25 p.m. **Session Two: Understanding the Dual Role of "Healthy Tissues" as Standards and "Normal" Reference Tissue**

10:55 a.m. – 11:10 a.m. **Fit-For-Purpose Approach to Normal Tissue**
Stephen Hewitt, M.D., Ph.D., Center for Cancer Research, NCI

11:10 a.m. – 11:25 a.m. **Reference kidney tissue from helathy controls**
Petter Bjornstad, M.D., Children's Hospital Colorado

11:25 a.m. – 11:40 a.m. **Ethical Considerations for Altruistic Kidney Biopsy**
Paul Kimmel, M.D., M.A.C.P., F.R.C.P., F.A.S.N., NIDDK

11:40 a.m. – 12:25 p.m. **Validation of Tissue Quality and "Health": A Combined View from the Kidney**

Agnes Fogo, M.D., Vanderbilt University
Sanjay Jain, M.D. Ph.D., Washington University
Jeffrey Spraggins, Ph.D., Vanderbilt University

Key Questions for Speakers

- What are the current best practices for obtaining "healthy reference tissue"?
- What quantitative metrics should be used to assess tissue quality?
- How "normal" is adjacent "normal tissue"?
- What are the limits of "no significant pathologic changes"?
- What are the pitfalls of subtraction analysis?
- What potential sources of variability do you worry about most?

12:25 p.m. – 12:45 p.m. **Break**

12:45 p.m. – 2:15 p.m. **Session Three: Developing Assay-specific Community Standards: Current State-of-the-art Standards: Lessons from the Bench**

12:45 p.m. – 1:00 p.m. Stephen Castellino (Xenovista LLC/GlaxoSmithKline)
—*Spatial Modalities*

1:00 p.m. – 1:15 p.m. Amanda Paulovich, M.D., Ph.D., Fred Hutchinson Cancer Research Center
—*Mass Spectrometry/Proteomics*

1:15 p.m. – 1:30 p.m. Charles Wang M.D., Ph.D., Loma Linda University
—*RNA/Molecular Assays*

1:30 p.m. – 1:45 p.m. Clay Davis, M.D.C.M., NIST
—*Metabolomics and Lipidomics*

1:45 p.m. – 2:00 p.m. Michael Rauchman, M.D., Washington University
—*Epigenetics*

2:00 p.m. – 2:15 p.m. Marc Salit, Ph.D., The MITRE Corporation
—Genomics

Key Questions for Speakers

- What standards have and have not worked?
- What quantitative metrics should be used to assess standard quality?
- What is the relative value of using reference tissue compared with fit-for-purpose standards? Does a role for both exist?
- What potential sources of variability do you worry about most?

2:15 p.m. – 2:45 p.m. **Session Four: Lighting Talks: Late-breaking Innovative Ideas. Moving the Field Forward and Developing Next Generation Fit-for-purpose Standards**

2:15 p.m. – 2:20 p.m. **Improving spatial N-glycomics for human kidney using reference tissue and robust quality control**
Chris Anderton, Ph.D., PNNL

2:20 p.m. – 2:25 p.m. **Nephrectomy Tissue as a Source for High Quality Reference Tissue**
Markus Bitzer, M.D., University of Michigan

2:25 p.m. – 2:30 p.m. **Long-Term Metabolomics Reference Material**
Goncalo Gouveia, Ph.D., University of Georgia

2:30 p.m. – 2:35 p.m. **Quality Control Metrics for Epigenetic Interrogation Technologies**
Michael Eadon, M.D. Indiana University

2:35 p.m. – 2:40 p.m. **Reference Material Requirements for High-Throughput Lipidomics using surface ionization.**
Aalim Weljie, Ph.D., University of Pennsylvania

2:40 p.m. – 2:45 p.m. **A Common Sample for Cross Platform, Multimodal Quality Control Standards of Spatial Resolution**
Melissa Farrow, Ph.D. Vanderbilt University

Key Questions for Speakers

- What are the gaps?
- Where are the opportunities?
- What are the new technologies that need to be improved or developed?
- What emerging technologies will require new types of standards?

2:50 p.m. – 3:50 p.m. **Session Five: Breakout Discussions**

With a background from prior sessions, discuss the current best practices and future needs. Where is technology development needed?

Key Question for Workshop Participants
What healthy reference tissue and/or standard should I use when I run an experiment in my laboratory next week?
Key Questions to Be Answered by Each Breakout Group

- What are the current best practices?
- What are the limitations to current best practices (pro and cons)?
- What improvements or new developments need to be made? What can be attained or achieved in one year? Five years?
- What community-wide experiments are needed to validate new technologies and standards?
- What potential sources of variability do you worry about most?

Breakout Group Discussions	**Discussion Leader(s)**
Group 1. Pre-analytics	Carolyn Compton
Group 2. Healthy Reference Tissues	Petter Bjornstad
Group 3. Transcriptomics	Sanjay Jain/Charles Wang
Group 4. Proteomics	Jeff Spraggins /Ben Neely
Group 5. Metabolomics and Lipidomics	Chris Anderton/Tony Dickherber
Group 6. Epigenomics	Michael Rauchman/Mike Eadon

3:50 p.m. – 4:00 p.m. **Break**

4:00 p.m. – 5:00 p.m. **Session Six: Breakout Reports (10 minutes each)**

Breakout Group Discussions	**Discussion Leader(s)**
Group 1. Pre-analytics	Carolyn Compton
Group 2. Healthy Reference Tissues	Petter Bjornstad
Group 3. Transcriptomics	Sanjay Jain/Charles Wang
Group 4. Proteomics	Jeff Spraggins /Ben Neely
Group 5. Metabolomics and Lipidomics	Chris Anderton/Tony Dickherber
Group 6. Epigenomics	Michael Rauchman/Mike Eadon

5:00 p.m. – 5:10 p.m. **Closing Remarks and Adjournment**