BioRepository & Precision Pathology Center Scope of Service

In order to aid research both inside and outside of Duke University Health System, the BRPC provides services in the areas of clinical trial support, research specimen collection, research histology, digital imaging, digital spacial profiling, nucleic acid extraction and quality control, research immunohistochemistry, and tissue microarray. This document will describe the services in detail per section.

1. Patient consent to Pro00035974
2. Demographic and clinical data annotation, organization, distribution
3. Tissue Procurement:
   1. Fresh tissue release
   2. Procurement, storage, processing, release of frozen tissue, OCT-embedded frozen tissue, FFPE tissue
4. Blood procurement
5. Support of individual investigator IRB protocols and clinical trials
   1. Fresh sample procurement and processing
   2. Access to archival paraffin tissue samples per IRB protocol specifications
6. Research Histology
   1. Routine paraffin processing, embedding, and sectioning
   2. Frozen Sectioning
   3. Hematoxylin and eosin (H+E)
   4. Special/histochemical stains: Congo Red, Cresyl Violet, Luxol Fast Blue, Mikat, Masson Trichrome, Martius Scarlett Blue, Oil Red O (frozen sections only), Periodic Acid Schiff, Periodic Acid Schiff-Diastase Digestion, Toluidine Blue

Immunohistochemical Staining: Routine single antibody IHC stain on paraffin section with standard DAB/brown color (chromogen of investigator’s choice available; price may vary)

Multiplex IHC for up to 4 antibodies using 4 different chromogens.

For a new antibody not in the current BRPC menu, we offer optimization and CAP-grade validation

Please see brpc.duke.edu/research-immuno-histo-chemistry to see currently available antibodies. Some antibodies include: (list each antibody on an individual line; only list the antibodies for which Emily can provide a validation document)

1. Digital Imaging

The BRPC digital imaging service offers high quality scanned images of histology slides. We have two state-of-the-art Aperio scanners: the GT-450 and AT-2 scanners, each of which is capable of scanning at an effective ‘40x’ resolution (0.26 um/pixel). Image quality has been demonstrated of sufficient quality for clinical archival purposes. Scanning may be performed on numerous staining modalities, including classic Hematoxylin & Eosin stain, trichrome stain, immunohistochemical stains, and other special stains. Our machines offer high throughput scanning capable of processing up to 81 slides per hour, depending on quantity of tissue per slide, enabling large volume projects for image analysis or development of artificial intelligence/machine learning tools.

In addition to these resources, the BRPC has availability of a machine with the Oncotopix suite of tools available from Visiopharm. These tools can be used for machine learning image analysis, including cell detection, positive cell quantification, and other feature identification from H&E, special stains or immunohistochemical stains. Tissue alignment tools can be used to co-register features between multiple slides, ideally from consecutively cut sections.

1. Digital Spatial Profiling

Digital spatial profiling (DSP) refers to the technology that allows for highly multiplexed protein or gene expression analysis within the two dimensional context of tissue architecture. Modern DSP platforms approach the resolution of single cell analysis, allowing for the elucidation of cellular heterogeneity within a tissue, organ, or disease process. There are two major spatial profiling platforms available in Duke CORE laboratories: the NanoString GeoMx and the 10x Genomics Visium. BRPC pathologists support Duke investigators’ DSP projects through experimental design suggestions, evaluation and identification of optimal tissues, and guidance with ROI selection.

Access to and use of the NanoString GeoMx Digital Spatial Profiler is available to any Duke investigator through the BRPC. The GeoMx DSP is housed within the BRPC laboratory space where all wet laboratory functions are performed, including tissue processing, slide cutting, slide staining, region of interest (ROI) selection, and oligonucleotide tag capture. Capture plates are then transported to other core laboratories for hybridization and detection on the nCounter instrument (for protein experiments) or for library preparation and next generation sequencing (for cancer transcriptome or whole transcriptome experiments). For protein expression experiments, the oligonucleotide tags with their quantitative counts are imported back into the GeoMx platform for integration with the slide images and ROI selections for quantitative spatially-resolved protein expression analysis. For transcriptome experiments, the DNA oligonucleotide sequences contain ROI indices mapping them back to their tissue location, an RNA target identification sequence matching them to their ISH probes, and a unique molecular identifier (UMI) to deduplicate reads. After sequencing, the FASTQ files are processed and converted to Digital Count Conversion (DCC) files on a dedicated server in the BRPC using the Nanostring GeoMx bioinformatics pipeline. The DCC files are then imported back into the GeoMx machine for investigator analysis. Investigators then have the option to access the GeoMx remotely to analyze their data using the NanoString Analysis Software Suite, or they can extract their data to analyze using their statistical software of choice. If additional biostatistics support is needed, investigators are referred to the Bioinformatics Service Center or the Genomic Analysis and Bioinformatics Core Facility, depending on whether their project is cancer-related or non-cancer-related.

Access to and use of the 10x Genomics Visium is available to any Duke investigator through the Molecular Genomics Core facility. The BRPC supports Visium experiments through tissue selection/evaluation, histology quality control, RNA quality control, and slide preparation. To learn more about the 10x Genomics Visium, click here.

1. Nucleic Acid Extraction and Quality Control

BRPC provides services of nucleic acid (DNA/RNA) extraction from frozen or FFPE tissues.

BRPC will consult with the scientist for their special interests, and will use tissue scrolls, macrodissection, pinpoint punching or Laser microdissection to collect tissues for the DNA/RNA extraction. We can also run DNA and RNA extractions simultaneously or separately per scientist`s request.

BRPC will use Qiagen AllPrep DNA/RNA kit, AllPrep FFPE DNA kit, AllPrep FFPE DNA/RNA kit and RNeasy kit for manually extracting nucleic acid, or use Maxwell RSC DNA/RNA kit (Promega) for automatic nucleic acid extraction.

BRPC will use Nanodrop to determine the concentrations of DNA and RNA samples, we also has Agilent Bioanalyzer that can be used for quality check (RIN and smear analysis) of the DNA/RNA samples.

1. Tissue Microarray

The TMA facility has the capability of producing blocks with cores ranging in size from 0.6mm to 2.0mm with the total number of cores per block dependent on the core size. Each microarray block is then subjected to a quality control test before releasing to the investigator.