

Duke Molecular Genomics Core 10x Genomics Xenium Project Workflow

Consult and/or Cost Estimate

- Email karen.abramson@duke.edu to request a cost estimate and/or consult meeting.
- Information required for estimate: sample species, input material (FFPE or fresh frozen tissue), how many samples, panels you want plus custom content and do you want data analysis.

Scheduling & Ordering

- Submit a service request in CoreResearch@Duke. We cannot order kits until this is received.
- If you are submitting an order for custom content, you must get a quote and work with 10x Genomics to design the panel.
- **If your project requires the assistance of a pathologist when choosing an ROI, your samples must be cut at the BRPC or BTBR.** You must reach out to either jadee.neff@duke.edu (BRPC) or diane.satterfield@duke.edu (BTBR) to begin their workflow.
- **Please note, any kits that we order for your project are non-refundable.**
- Email karen.abramson@duke.edu to schedule your RNA QC and your full 10x run.

Preparing for your run

- For your planned 10x run, fill out and email us the "New Project Summary and Sample Submission" forms.
- If a pathology core is cutting your tissue, once you've told them which samples to use and they've performed a QC, they will work with the MGC for subsequent steps.
- RNA QC is optional but recommended! Performing an RNA QC provides an opportunity to choose the optimal samples to use for the assay.
- If we are cutting your tissue, our team will reach out to arrange for sample drop off.

Run day

- We will run the assay and inform you of any issues that may arise.
- Once processing is complete, we will update you.

Post-imaging at Duke

- If we are providing data analysis, we will download the data and begin processing.
- The data analysis team will contact you for more details regarding your analysis plan.

Duke Molecular Genomics Core

10x Genomic Xenium Sample Preparation Guidelines

It is essential that you follow the sample preparation protocol provided by 10x Genomics® and the MGC.

The 10x Genomics® Xenium assay uses both fresh frozen and FFPE tissue placed on the surface of a Xenium slide. An RNA QC is not required but could be a good indicator as to how well a tissue may perform or be a good metric to have for troubleshooting later. An H&E and/or a Dapi stained tissue could help determine quality of morphology and nuclei intactness.

The MGC does not have a pathologist on staff, any project requiring a pathologist to identify a region of interest must either be cut in your own lab or work with a pathology core. The BRPC or BTBR has been trained to cut slides for Xenium and have workflows in place.

BRPC (Jadee Neff: jade.neff@duke.edu)

BTBR (Diane Satterfield: diane.satterfield@duke.edu)

The quality of RNA in a sample is greatly affected by collection processing, storage and age of sample. It is recommended for new tissue collection to reduce time between collection of tissue and preservation. Extended time between collection and preservation will greatly reduce quality. Always keep fresh tissues on ice while processing. FFPE samples should be stored at 4C to best preserve quality.

It is recommended to optimize freezing protocols to reduce cracking, crystallization or tissue distortion. Following 10x's recommended guidelines for freezing is a good place to start.

The best way to ensure good quality data is to strictly follow the provided sample preparation guidelines.

The full 10x Genomics' Tissue Preparation Guide can be found here:

FFPE:

<https://www.10xgenomics.com/support/in-situ-gene-expression/documentation/steps/tissue-prep-ffpe/xenium-in-situ-spatial-profiling-for-ffpe-%E2%80%93-tissue-preparation-guide>

Fresh Frozen:

<https://www.10xgenomics.com/support/in-situ-gene-expression/documentation/steps/tissue-prep-fresh-frozen/xenium-in-situ-spatial-profiling-for-fresh-frozen-%E2%80%93-tissue-preparation-guide>

Please don't hesitate to ask questions! We're here to help! Please email DMPI-MGC@dm.duke.edu or karen.abramson@duke.edu.