

Duke Molecular Genomics Core
10x Genomics Visium 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: assay type, sample species, input material (FFPE or fresh frozen tissue), how many samples, how many batches, and do you want data analysis.

Scheduling &
Ordering

- Submit a service request in CoreResearch@Duke. We cannot order kits until this is received.
- 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 highly recommended! Performing an RNA QC provides an opportunity to choose the optimal samples to use for the assay. Visium FF requires a RIN of at least 7.0 and FFPE requires as low as 30% DV200 for the CytAssist workflow.
- If we are cutting your tissue, our team will reach out to arrange for sample drop off.
- If your project is fresh frozen, we will perform the tissue optimization test first.

Run day

- We will run the assay and update you with any issues that may arise.
- Once processing is complete, we will contact you to verify the sequencing plan.
- Libraries will be either transferred to the Duke Sequencing Core or back to you.

Post-sequencing
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.
- It is important that you download the raw data, regardless of whether we are providing the analysis or you are doing it on your own. We will only store raw data for 3 months.

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10x Genomic Visium Sample Preparation Guidelines

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

The 10x Genomics® Visium fresh frozen assay uses poly-a capture technology to bind mRNA to the surface of a slide. How far the RNA travels from its original position is affected by how long the tissue is permeabilized. In order to get the most accurate spatial information and high-quality data, a tissue optimization assay should be performed to determine permeabilization time. Ideally this test is done on all tissues that may differ in pathology or phenotype. If this is impractical and you request to only test one representative tissue, we cannot guarantee the permeabilization will be optimized across the range of phenotypes/disease pathology in other tissues and this **may affect the data**.

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 Visium 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:

CytAssist FFPE:

<https://www.10xgenomics.com/support/spatial-gene-expression-ffpe/documentation/workflows/cytassist-ffpe/steps/tissue-prep/visium-cyt-assist-spatial-gene-expression-for-ffpe-tissue-preparation-guide>

Fresh Frozen:

<https://www.10xgenomics.com/support/spatial-gene-expression-fresh-frozen/documentation/steps/tissue-prep/visium-spatial-protocols-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.