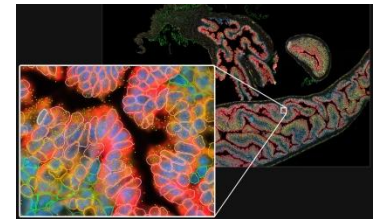


Xenium



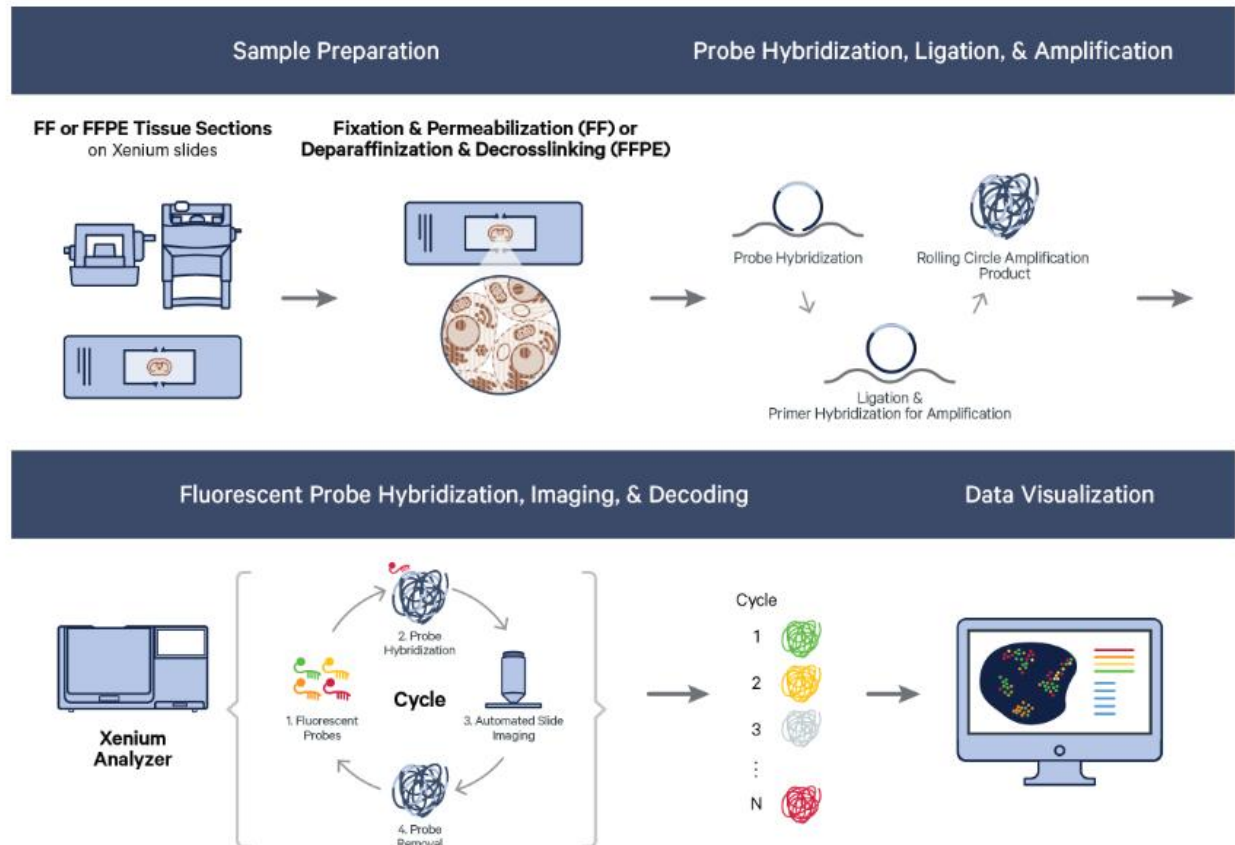
Xenium by 10X Genomics is a single-cell spatial analysis platform that allows researchers to analyze the expression of up to 1,000 genes in individual cells within their spatial context in tissue sections. It combines single-cell RNA sequencing, spatial transcriptomics, and immunofluorescence imaging to provide a comprehensive understanding of the molecular and cellular structure of tissues. Xenium can detect and analyze rare cell populations, such as stem cells or immune cells, in complex tissues.

TRIP performs **process and embedding, RNA extraction***, & **Xenium slide histology** in the workflow.

GEC performs all subsequent steps.

**For clients running samples on Xenium instruments outside the GEC facility, TRIP will provide RNA QC for all samples.*

Workflow



Sample Preparation

Formalin-fixed paraffin-embedded (FFPE) Tissue

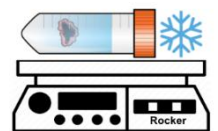
Improper fixation can lead to:

- Poor transcript density
- Nuclei degradation
- Reduced decoding performance
- Poor transcript assignment to cells
- Degradation of proteins, nucleic acids, & lipids
- Fragmentation of nucleotides
- Presence of artifacts such as irregularly shaped cells & irregular chromatin patterns
- Overstained cytoplasm (eosin)
- Autolysis artifacts, separation of epithelium from connective tissue
- Loss of structural integrity of molecular features
- Oxidation of lipids
- Excessive cross-linking and decreased antigenicity
- Tissue hardening making sectioning difficult



Tissue Size and Fixation:

- Tissue should be no larger than a standard cassette
- Thick sections will not allow for adequate penetration of fixative
- Handle gently to avoid damaging tissue structure
- Avoid areas of hemorrhage from surgical trauma or electrocautery
- Proceed quickly from collection to NBF, **minimizing ischemia**
- 10% Neutral Buffer Formalin (NBF)** is recommended for fixation
- Fully submerge in a fixative to tissue ratio: 20:1
- Fixation process should be carried out at **4° Celsius** for **~16-24 hours**
- Lightly agitate tissue throughout fixation process
- Leaving samples too long in fixative can lead to over fixation
- Fixation penetration rates depend on tissue thickness & may need optimization
- Transfer sample to **70% EtOH** for short-term storage (potentially up to 3 months)
- Refresh EtOH weekly to ensure concentration remains at 70%
- *Preferably bring samples to TRIP immediately for process & embedding



Fixed Frozen (FF) Tissue

Xenium In Situ Gene Expression can theoretically analyze mRNA in tissue sections derived from fixed frozen tissue samples, but is **officially unsupported by 10X Genomics**.



10X Genomics cannot guarantee assay performance for fixed tissues embedded in **OCT**, therefore clients should proceed at their **own risk**. 10X Genomics does not have additional details from internal experiments and **will not provide replacement reagents** for poor assay performance.

Sample prep and guidance provided by 10X Genomics follows guidelines adapted from Visium CytAssist Spatial Gene Expression for Fixed Frozen – Tissue Preparation Guide.

https://cdn.10xgenomics.com/image/upload/v1692048186/support-documents/CG000579_Demonstrated_Protocol_XeniumInSituProtocolsFF_TissuePreparationGuide_Rev_C.pdf

<https://kb.10xgenomics.com/hc/en-us/articles/17968908868877-Are-fixed-tissues-embedded-in-OCT-compatible-with-Xenium>

Prior to collecting samples, discuss your project intentions with the Gene Expression Center (GEC).

Email:

gecinfo@biotech.wisc.edu

OCT Before Freezing



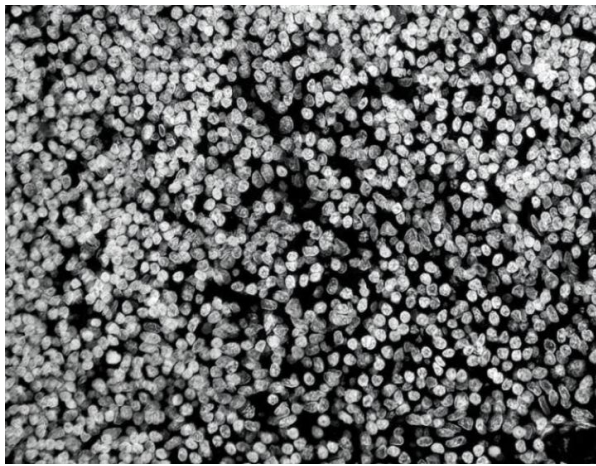
Frozen OCT



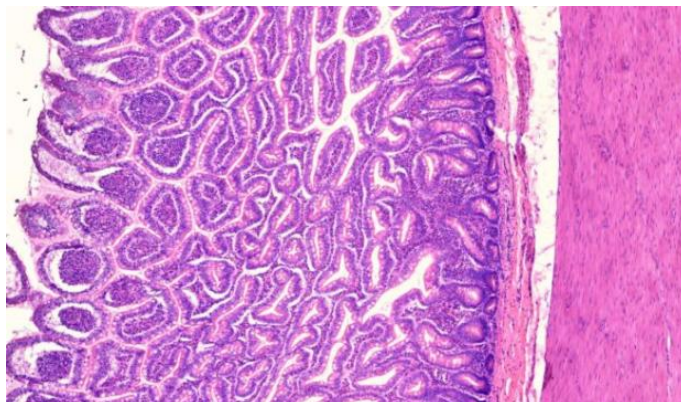
Sample Quality Control

10x Genomics recommends one or more QC assessment methods from the following options:

1. **DAPI staining:** TRIP will stain 1 DAPI slide from each of your blocks. DAPI is a nuclear stain that enables the assessment of nuclei quality, a predictor of assay success. However, good nuclei quality is not an absolute determinant of assay success.



2. **H&E staining:** TRIP will stain 1 H&E slide from each of your blocks. H&E staining allows for the examination of tissue morphology, looking for signs of tissue damage, artifacts, and proper fixation.



https://www.ihappysci.com/product/human-ileum-cross-section-histology-slides/?srsltid=Afm8OorkZSWU3S0Yil9pTh4FBVmgGmpSNrBnkGq_IQoS-Gp7Ht0Cyr

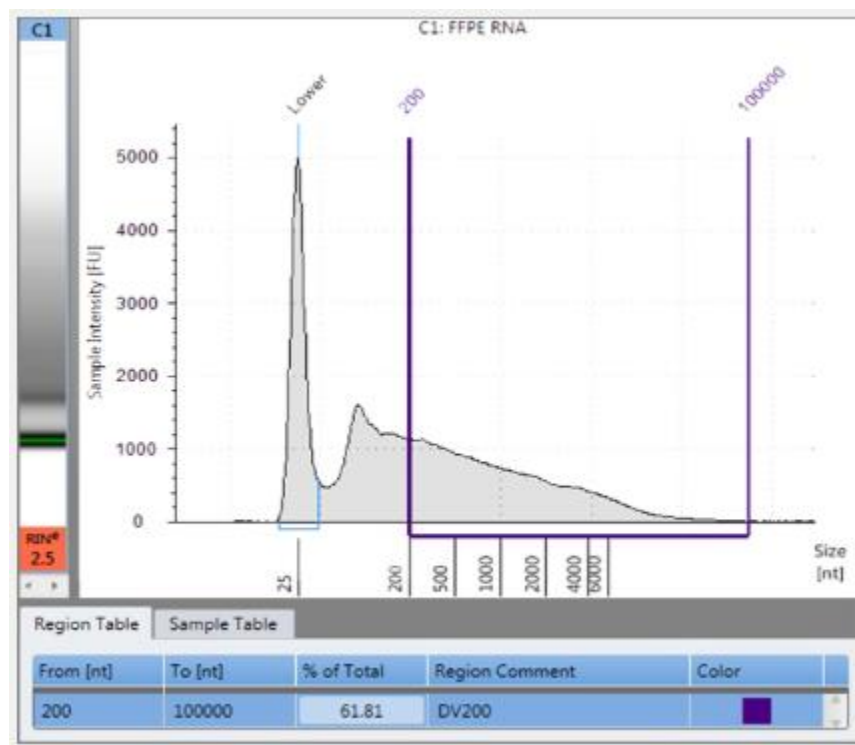
3. **DV200:** A DV200 reading assesses RNA integrity, where the DV200 score indicates the percentage of RNA fragments over 200 nucleotides in length. A DV200 score of less than 30% is correlated with low transcript density. TRIP will collect enough tissue from each sample to extract RNA for you to submit to GEC for Xenium RNA QC. They will provide concentration, A260/280, and DV200 reading.

TRIP's RNA extractions are done via **Maxwell CSC/IVO automated instrument**.
The samples are eluted into **nuclease-free water** and have been **DNAsed**.

TRIP will provide RNA in **strip tubes**, each tube labeled with the sample name, and each strip labeled with the PI and date per GEC recommendation.

Alternatively, TRIP will collect the tissue, and you can extract the RNA yourself.

Transport of RNA to GEC is to be done by the client.



<https://www.agilent.com/en/promotions/tapestation-dv200-determination>

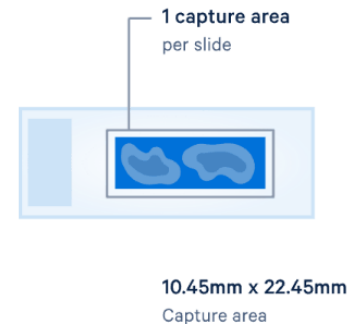
DV200=61.8%

Slide Capture Area

The sample area can accommodate as many tissue sections as can fit within the space without overlapping (including paraffin or OCT).

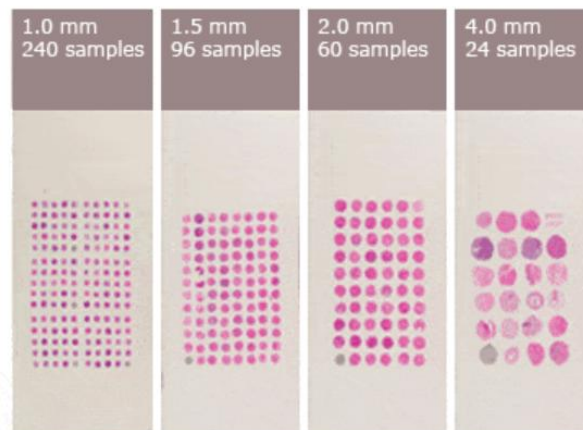
Micro Array = Tissue array blocks with >100 samples in the array

Macro Array = Tissue array blocks with ~10–75 samples in the array



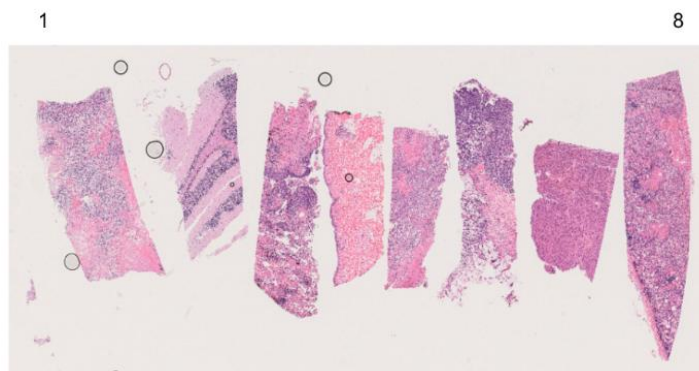
TRIP can create **Micro Arrays** using **biopsy punches** in the following sizes:

1.0mm	2.0mm	5.0mm
1.5mm	4.0mm	6.0mm



<https://www.biochain.com/blog/the-tissue-microarray-tissue-analysis-from-research-to-treatment/>

A **Macro Array** can be created with **larger pieces** of tissue placed into a single **FFPE block**.



1. Breast
2. Brain
3. Lung
4. Skin
5. Pancreas
6. Colon
7. Liver
8. Kidney

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GEC Contacts: GEC: gecinfo@biotech.wisc.edu