

TRIP's Visium FFPE with CytAssist Workflow

- 1- Client provides TRIP with information regarding histology and sample QC needs*
 - a. What is the tissue type? (Compatibility with Visium should already have been confirmed by GEC)
 - b. Do you need tissue embedding or are samples already in FFPE blocks? Tissue block should have a minimum of 30 μ of target tissue available for sectioning.
 - c. Do you have freshly cut standard H&E slides corresponding to each of your samples? If not, TRIP will create these.
 - d. Do you need a pathologist (highly recommend) to circle the ROI on standard H&E slides? Otherwise, provide standard H&Es with circled ROI for each sample.
 - e. Does TRIP need to do FFPE RNA extractions for QC? Alternatively, you would perform the extractions yourself.
 - f. How many total samples (blocks)?
 - g. Which Visium CytAssist slide format? (6.5mm or 11mm FOV format)

*Refer to GEC (gecinfo@biotech.wisc.edu) for: Visium Project Design meeting, Visium CytAssist technical questions, sample QC results, scheduling of project and legal documents if applicable.

Note 1: Only one section can be placed per slide to produce Visium H&E slide.

Note 2: ROI will be selected on a standard H&E slide ahead of time by investigator/pathologist. Visium H&E slide is the final slide that will be imaged on Aperio and used on the CytAssist for the Visium experiment.

Note 3: If you are submitting human FFPE tissue, we will need approved IRB documentation before commencing with the project.

Note 4: If you are an external/industry investigator, you need to have all MTAs, DTAs and other legal documents before commencing project. Coordinate with GEC.

- 2- Client submits Visium request in TRIP's iLab platform. The above questions will be filled out in TRIP's iLab form
 - a. [TRIP's iLab site](#)
 - b. To setup an iLab account, follow instructions [here](#). External clients can pay by credit card or check.
- 3- Client provides H&Es with ROIs circled or if the client needs assistance, they will set up an appointment with a pathologist to view slides, discuss their needs, and circle ROIs.
- 4- RNA extractions for QC are performed by client or TRIP, after which the client will deliver them to GEC.
- 5- GEC will inform the client if their samples passed QC and have met Visium standards to move forward. Then the client will be notified when their project is ready to begin.
- 6- TRIP will section the FFPE block(s) and center the selected ROI area within CytAssist FOV.
 - a. If the client is providing their own freshly cut slides, they need to include an ROI-circled standard H&E and minimum of 2 unstained slides per sample. Samples provided in this manner must also pass QC testing.
- 7- Visium H&E slide will be prepped, coverslipped, Aperio 20X scan, coverslip removed, and slide baked for 3 min.
- 8- Slides may be stored at 4°C for up to 2 weeks.
- 9- Client will be contacted to pick up slides and coordinate a drop off with GEC.

GEC Contacts:

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