

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? (Prior confirmation of tissue compatibility with Visium by GEC)
 - b. Is tissue embedding needed or are samples already in FFPE blocks? Tissue block requires a minimum of 30µ target tissue for sectioning.
 - c. Do you have freshly cut standard H&E slides corresponding to each of your samples? If not, TRIP will create.
 - d. Provide standard H&Es with circled ROI for each sample. Pathologist assistance is highly recommended. See TRIP for Pathologist contacts to view slides & discuss needs.
 - e. Does TRIP need to do FFPE RNA extractions for QC? Alternatively, you will 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, scheduling of project, & 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 & 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 & 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 check.
- 3- RNA extractions are performed by the client or TRIP, after which the client will deliver RNA to GEC. GEC will inform the client if their samples passed QC, have met Visium standards, & when the project will begin.
- 4- TRIP will section the FFPE block(s) & 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.
- 5- Visium H&E slide will be prepped, coverslipped, Aperio scan @ 20X, coverslip removed, & slide baked for 3 min.
- 6- Slides may be stored at 4°C for up to 2 weeks.
- 7- Client will be contacted to pick up slides from TRIP and coordinate a drop off with GEC.

TRIP Contacts: TRIP Lab: trip@pathology.wisc.edu Toshi Kinoshita sskinosh@wisc.edu Rebecca Baus rmbaus@wisc.edu
Karla Esbona (TRIP Director): kesbona@wisc.edu

GEC Contacts: GEC: gecinfo@biotech.wisc.edu Harrison Pantera: pantera@wisc.edu Bridget Ladell: bladell@wisc.edu
Sandra Splinter Bondurant (GEC Director): sandrasb@biotech.wisc.edu

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