VAI Spatial Transcriptomics Policies

Please thoroughly review these policies before submitting samples. If you have any questions regarding this policy or any other aspect of next-generation sequencing or spatial transcriptomics, please contact us at genomics@vai.org.

Sample Submission:

1. Before submitting an initial project, VAI researchers must meet with Genomics Core staff to discuss project goals and details of starting material. To initiate this meeting, email spatial@vai.org.
2. Projects are received on a first-come, first-served basis and will not be entered into the project queue until samples are in possession of the Genomics Core and a project submission form is completed on our website (https://vari.my.site.com/submission/s/login/). Please get in touch with the Genomics Core directly if any extenuating circumstances are associated with a project being submitted.
3. Once samples are entered into the queue, the Core will provide a tentative project timeline. We strive to honor this timeline; however, issues beyond our control may delay the project. In the event of a delay greater than a week, the Core will email the customer to inform them of an updated run date.
4. After samples are processed, Core personnel will initiate a CrossLab request for the project. Please review and authorize the project if everything looks correct.

PHI: VAI Genomics Core does not accept samples or documentation that contain PHI. In the unlikely event that PHI is transferred incidentally, the Core will inform the submitting lab within 5 business days of becoming aware of the disclosure and will subsequently destroy any identifiable PHI (including samples and ePHI).

Customer Responsibilities:

- Dropped-off samples must be prepared to VAI specifications unless the Core Director has granted previous permission. A summary of submission requirements can be found on pg. 3 of these policies.

VAI Deliverables:

- For projects that require sequencing, a minimum of the requested number of bases for the entire project, 80% bases Q30 (99.9% accuracy) or greater, will be delivered in FASTQ format.
- Reads will not be trimmed for adapter read through.
- Data are delivered in FASTQ format within 5 business days of run finish. Further analysis may be contracted separately with the Bioinformatics and Biostatistics Core (BBC).
- Libraries that do not meet these specifications will be re-run as soon as possible; however, there may be a wait for a run that will meet or exceed the requested parameters. Libraries may not be run on the same length flowcell for expediency.
- All additional required files for spatial projects will be delivered within 5 business days of the run finish; the additional file types are subject to the type of spatial project. See pg. 3 of these policies

Exceptions:

While we will make every effort to create successful spatial projects with high-quality data output, we cannot be responsible for improperly prepared samples. Therefore, we make **no guarantees** on the quantity and quality of data generated from the project if:

1. The user prepares the libraries/slides, as we have minimal control over quality.
2. Samples that do not meet VAI minimum submission requirements are submitted.
3. Reagents/slides are purchased/delivered to anyone other than directly to the Genomics Core.
4. Species are sequenced for which no reference genome is available.

If your data do not meet these criteria, or if you have other questions/concerns regarding your data, please contact us at genomics@vai.org to address your concerns.

Minimum and Optimal Sample Requirements:

For spatial projects, we highly recommend utilizing the histology services of an accredited Core, such as the Pathology and Biorepository Core at VAI (histopathology@vai.org) to prepare your tissue to the approved specs of the spatial assay. You will be emailed if the following initial QC requirements are unmet. You may either resubmit samples of sufficient quality and quantity or proceed with the existing samples. Should you proceed with samples that do not meet our requirements, the samples will be run AS IS, and we will make no assurances on the quality of downstream data. For spatial options not listed or for further clarification, please visit VAI's Spatial Collaborative Services website or email spatial@vai.org. **All spatial projects require an initial meeting with a genomics/imaging core associate before project initiation.**

Nanostring GeoMx projects

- Sample types: Fresh frozen, FFPE, or OCT from mouse or human tissue only.
- Fresh frozen samples should be flash frozen in liquid nitrogen cooled isopentane as soon as possible after collection. Optimally embedded in OCT or CMC at the same time or afterward.
- For FFPE samples, please follow standard formalin fixation and embedding protocols. Samples should be stored at 4C post fixation to preserve RNA quality.
- Optimal tissue thickness is 5um, and should be sectioned onto positively charged slides no more than 2 weeks prior to running the assay
- A morphology check is required at least 1 week before the collection run.
- A minimum of 100-200 cells per ROI is recommended for RNA assays and 50 cells per ROI for protein assays.
- A minimum of 6 ROIs per type is recommended for meaningful statistical analysis.
- Tissue must fit within a 40mm x 17mm slide region, please see the example schematic provided by Nanostring.
- Sequencing will be performed on the un-normalized library pool/s to the recommended depth (100/ um^2 collection area) unless otherwise specified.
- FASTQ files, DCC zip folder, Pkc files, initial data set from Nanostring GUI (excel), and the lab worksheet that contains all the ROI information will be delivered within 5 days post project completion.

10X Genomics Visium projects (std)

- Sample types: Fresh frozen samples from human or animal origin, FFPE samples from mouse or human.
- For Fresh Frozen samples, flash freeze in liquid nitrogen cooled isopentane as soon as possible after collection. Optimally embed in OCT or CMC at the same time or afterward. Tissue sections should be <6.5mm x 6.5 mm, and optimal thickness is 10um. RIN quality should be >7.0
- For FFPE samples, follow standard formalin fixation and embedding protocols. Samples should be stored at 4C post fixation to preserve RNA quality. Tissue should be < 6.5mm x 6.5mm or <11mm x 11mm to be compatible with visium spatial slides. Optimal tissue thickness is 5um. RNA quality DV200 >50%.
- 55 um capture spots, 100um center to center. Each spot will capture 1-10 cells.
- Sequencing depth will be calculated by determining the tissue coverage area x 25,000 read pairs/spot unless otherwise specified.
- FASTQ files, probe set, any sample indexing, slide ID and capture area and any microscope images will be provided within 5 days post project completion.

10X Genomics Visium HD projects

- Sample types: FFPE samples from mouse or human.
- For FFPE samples, follow standard formalin fixation and embedding protocols. Samples should be stored at 4C post fixation to preserve RNA quality. Tissue should be < 6.5mm x 6.5mm to be compatible with visium spatial slides. Optimal tissue thickness is 5um.
- Continuous lawn of 2 x 2 um barcoded squares with no gaps.

- Sequencing depth will be calculated by determining the tissue coverage area x 275,000 read pairs unless otherwise specified.
- FASTQ files, probe set, any sample indexing, slide ID and capture area and any microscope images will be provided within 5 days post project completion.

Lab Prepared Libraries:

- At least 10uL of library at ≥2ng/ul, measured by fluorometry only, in 10mM Tris pH 8.0 or water. EDTA should be avoided as it retards downstream sequencing reactions.
  - More material may be needed for libraries requiring higher amounts of reads, such as full flow cell lanes.
- Provide all index *SEQUENCES*, as the same index number from different library preparation kits do not always refer to the same sequence. Sequences are provided by the vendor, usually either in an appendix or separate supporting table.
- Index sequences should be provided in the following orientation regardless of intended sequencer to be used (contact the Genomics Core if you are unsure of proper orientation):
  - i7: i7 Bases for Sample Sheet'
  - i5: 'Reverse Orientation' – some vendors will list which instruments use a specific orientation; the Reverse Orientation will have NovaSeq 6000 v1.5 listed.
- Projects containing libraries with incorrect indices provided will be subject to a $240 charge for additional processing and will cause delays in data release, including possible resequencing.
- Libraries containing UMIs should note the length and location of these sequences on the project page. The VAI Genomics Core is not responsible for UMIs not sequenced due to the absence of notification or inaccurate notification of UMI placement within the library.
- If possible, please provide an expected size for your library. A gel image is not necessary but can be helpful.
- Charges for lab prepared libraries will include the cost of pre-sequencing Bioanalyzer, fluorometric quantification, and qPCR QC.

Sample Delivery and Labeling:

Tissue delivery should be arranged with the Histology group following their rules and regulations. If submitting already prepared slides with sectioned tissue, please place them in 50ml Eppendorf tubes or an appropriate alternative with desiccant. Arrange for a drop off time so samples are handed directly to a Genomics Core associate or gain access to the spatial room for drop off in the 4C fridge. Notify the Genomics Core of sample drop-off as well as provide any other supplementary files (image files, date of sectioning, etc.). **Drop-off of already prepared slides must be arranged beforehand (2-3 weeks).** Tubes/plates must be labelled with the project number from our submission portal (PRxxxxxx) and sample name (matching what is submitted in the portal). Sample names will be 6-8 characters in length and must be composed only of alphanumeric characters. Any spaces, dashes, underscores, or special characters will be removed, as they are not compatible with the sequencing software.

Libraries should be submitted in 1.5mL microcentrifuge tubes, unless there are ≥24 in which case they should be submitted in a full skirted 96-well PCR plate (such as Eppendorf twin.tec). Do not submit libraries in strip tubes or you will be asked to transfer your samples and resubmit. If submitting in a PCR plate, place samples in column orientation (A1, B1, C1, etc.). Tubes/plates must be labelled with the project number from our submission portal (PRxxxxxx) and sample name (matching what is submitted in the portal). Sample names will be 6-8 characters in length and must be composed only of alphanumeric characters. Any spaces, dashes, underscores, or special characters will be removed, as they are not compatible with the sequencing software.

Data Storage:

Deliverable sequence data will be stored in an HPC download directory accessible by your lab. Data for labs outside of VAI will be stored on a lab-specific directory shared on Globus. Files will be removed by the Genomics Core after 2 weeks due to space considerations; please be prompt in downloading your data. Raw,

binary output from each sequencing run will be stored by the VAI Genomics Core for 60 days. If you wish to have a copy of this data, you **must** contact the Core Director at genomics@vai.org within 1 month of the beginning of your sequencing project to facilitate the transfer.

Leftover Samples:
Remaining slides will be returned after project completion if applicable, customers will be notified when samples are ready for pick up. Alternatively, a notification of disposal will be sent via email both 2 weeks and 1 day prior to disposal, after which samples will be discarded if the lab has not contacted the Genomics Core. Please contact genomics@vai.org for pick-up arrangement. Libraries generated by the VAI Genomics Core will be stored indefinitely.