GENOMICS CORE ILLUMINA SEQUENCING POLICIES

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

SAMPLE SUBMISSION

- Prior to submitting an initial project, VAI researchers will be required to meet with Genomics Core staff to discuss sequencing goals and details of starting material.
- Projects are received on a first-come, first-served basis, and will not be entered into the project queue until samples and a completed submission form are in the possession of the Genomics Core. Once samples are entered into the queue, the Core will provide a tentative project timeline. We strive to honor this timeline; however, there are issues beyond our control that may delay the run. In the event of a delay greater than a week, the Core will email the customer to inform them of an updated run date.
- After samples are received, Core personnel will initiate an iLab request for the project. Please review and authorize the project if all looks correct.

CUSTOMER RESPONSIBILITIES

- Dropped-off samples must be prepared to the Core's specifications, unless previous permission has been granted by the Core Manager. A summary of submission requirements can be found on page 2 of these policies.
- The organism name and sequence of a phylogenetically analogous reference genome (e.g., hg19 for *Homo sapiens*) must be supplied, if available.

CORE DELIVERABLES (PER FLOWCELL)

- NextSeq: Minimum 380M reads (or read pairs) passing filter, 80% bases Q30 (99.9% accuracy) or greater, delivered in FASTQ format.
- A FASTQC report will be delivered for each library. These files provide overviews of run quality. Reads will not be trimmed for any reason.
- Data are delivered in FASTQ format within five business days of run finish. Further analysis may be contracted separately with VAI's Bioinformatics and Biostatistics Core.
- Libraries that do not meet these specifications will be re-run at the earliest possible date; however, there may be a wait for a run that will meet or exceed the requested parameters.

EXCEPTIONS

While we will make every effort to create successful sequencing runs 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 Illumina sequencing if:

- Libraries are prepared by the user, as we have minimal control over quality.
- Samples are submitted that do not meet VAI minimum submission requirements.
- Non-Illumina barcoding methods are used. Barcodes placed at the 5'end of the sequence with no redundancy are especially prone to read errors.
- Species are sequenced for which there is no reference genome 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.

MINIMUM AND OPTIMAL SAMPLE REQUIREMENTS

If the following initial QC requirements are not met, you will be informed via email. You may elect to either resubmit samples of sufficient quality and quantity or proceed with the existing samples. Should you choose to proceed with samples that do not meet our requirements, the samples will be run **AS IS**, and we make no assurances on the quality of downstream data.

gDNA

- 100 ng–3 µg of high-quality, high-molecular weight gDNA in 10mM Tris pH 8.0 or water.
- Quantify DNA by fluorometric methods if possible. If quantifying by spectrophotometer, A_{260}/A_{280} should be ~ 1.8. deviations from this number suggest inaccurate quantification.
- Provide a gel image to assess DNA quality — a good image will have a distinct band above 10kb with no smearing.
- Column-based cleanups or ethanol precipitation are strongly recommended prior to drop off, especially for organic extractions.

ChIP

- 10 ng minimum, 30 ng optimal, post-IP DNA sheared to 200–600 bp. DNA sheared to >1000 bp will not be accepted, as it is not compatible with the sequencer.
- Quantify DNA by fluorometric methods if possible. Do not quantify IPs by spectrophotometer.
- qPCR on a known target is recommended to verify enrichment, as is submitting an input sample as a normalization control.

RNA

- 1 µg of total RNA or 100 ng of mRNA in10mM Tris pH 8.0 or water.
- A_{260}/A_{280} and A_{260}/A_{230} ratios should be ≥1.8 in the range of 1.8 to 2.2. Ratios ≤1.8 may indicate protein and/or organic contamination.
- Column-based cleanup is strongly recommended, especially for organic extractions, as well as assaying ribosomal peak integrity on an Agilent Bioanalyzer PicoChip.
- Prokaryotic total RNA must be ribosomally reduced by the submitting lab prior to sample submission.

Lab-Prepared Libraries

- At least 10 µL of library at >5 ng/µL, measured by fluorometry only, in 10mM Tris pH 8.0 or water. EDTA should be avoided as it inhibits downstream sequencing reactions.
- Provide all index **SEQUENCES** as the same index number from different library preparation kits do not always refer to the same sequence.
- 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 and qPCR QC.

SAMPLE LABELING

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. Files will be removed by the Genomics Core after two weeks due to space considerations; please be prompt in downloading your data. Raw, binary output from each sequencing run will be stored by the Genomics Core for 90 days. If you wish to have a copy of this data, you **must** contact the Core Manager at genomics@vai.org **within one month of the beginning** of your sequencing project to facilitate the transfer.

LEFTOVER SAMPLES

Any remaining samples will be stored for 90 days after project completion. Customers are welcome to collect any leftover materials during this period, after which, they will be discarded without notification. Please contact genomics@vai.org to arrange pick-up.