University of Pittsburgh

High Throughput Genomics Core (HTGC)

SAMPLE SUBMISSION REQUIREMENTS

We accept premade libraries and purified DNA/RNA for library preparation. We also accept a variety of samples for DNA/RNA extraction including fresh frozen tissues/cells, blood, OraGene kits for saliva, lung brush, cell pellets, and other samples.

See below for details regarding some of the more common sample types submitted. If you have questions about a sample type, not listed please contact us at HTGC@pitt.edu.

A. Whole Blood

A minimum 3 mL of whole blood in EDTA or ACD tubes (heparin is not desired, please confer with the laboratory prior to submission), ship with cold not frozen gel-pack to minimize heat damage. If your sample amount should be limited for any reason, you may submit a reduced sample volume. Please contact us to discuss available options. **DO NOT FREEZE**. Submission of specimens older than 7 days may not be acceptable and may be rejected.

B. Saliva

Saliva or buccal wash using Oragene collection kits shipped at ambient temperature with cold, not frozen, gel-pack if temperature along transit route is anticipated to be above 75°F.

C. FFPE

Slides or FFPE blocks should be shipped with frozen gel-pack if temperature along transit route is anticipated to be above 75°F. To perform microdissection, submit 1 H&E with area selected and 10 blanks. Scrolls are also acceptable, five 10µm scrolls in 1 tube per block, or three 20µm scrolls.

D. Fresh/Frozen Tissue

Store tissue at –80°C and transport on dry ice to minimize freeze thaw cycles. We recommend 10mg tissue per extraction (for instance submit >20mg for both DNA and RNA extraction). Contact us to discuss submission of smaller pieces of tissue. Tissue stored in RNAlater or RNAprotect is also accepted.

E. Cell Pellets

Cell pellets ranging from ~100K-1M cells are recommended for extraction. Cell pellets purified by Ficoll gradient, or differential lysis are preferred for cells collected from blood samples. They may be submitted as a pellet or suspended in PBS or RPMI and shipped with cold, not frozen, gel-pack if temperature along transit route is anticipated to be above 75°F for DNA. Frozen cell pellets either dry or in freezing media may be shipped frozen on dry ice.

F. Purified DNA/RNA Samples for Library Preparation Requirement

Recommended to submit in Bio Rad Hard-Shell 96-Well PCR Plates, low profile, thin wall, skirted HSP9601, as they are used with our laboratory robotics to process the samples OR use 1.5 mL Lo-bind microfuge tubes. We can provide tubes/plates as needed; you can pick them up when you are ready to submit. Tubes should be submitted in a box/rack in the order as listed on the submission form (left to right, top to bottom, not randomly placed, and not loose/unracked). Strip tubes are not recommended as they are difficult to label and break more frequently. Sample quantification is recommended prior to submission. Samples should be dissolved in EDTA-free solution such as nuclease-free water or 10 mM Tris-Cl (pH 8.5). Extracted RNA must be shipped frozen on dry ice. Our standard library prep for both RNA and DNA uses minimum 500ng input material.

NOTE: Quantify the DNA and RNA concentrations using a fluorescence-based method such as Qubit or PicoGreen. If you cannot do fluorometry, please send us 2X the minimum amount that NanoDrop determines. Concentrations determined by an absorbance-based method such as NanoDrop are variable and often overestimate the amount of nucleic acid. We may request more sample amount than we need to allow for pre-library preparation QC and any repeats that we may need to perform. If your sample amount should be limited for any reason,

you may submit a reduced sample volume. The samples *do not need* to fall within the recommended ranges, but if required to dilute samples based on minimum volume, ranges are below. Please contact us to discuss available options.

*Concentrations are listed for fluorescence-based measurements.

G. Prepared Libraries Requirement

Please submit samples in Bio Rad Hard-Shell 96-Well PCR Plates, low profile, thin wall, skirted HSP9601, as they are used with our laboratory robotics to process the samples. We can provide plates as needed; you can pick them up when you're ready to submit. Lo-bind microfuge tubes, 1.5 mL or 0.7mL, are also acceptable. Each tube must be clearly labeled numerically with the sample name. Tubes should be submitted in a box/rack in the order as listed on the submission form (left to right, top to bottom, not randomly placed, and not loose/unracked). Strip tubes are not recommended as they are difficult to label and break more frequently. Sample quantification is recommended prior to submission. Samples should be dissolved in EDTA-free solution such as nuclease-free water or 10 mM Tris-Cl (pH 8.5).

Please submit undiluted libraries. In general, libraries that pass the recommended or anticipated yield ranges from library prep kits will be sufficient for sequencing. QC (Fragment Analyzer and fluorescent quantification, optional qPCR) will be performed on all the libraries submitted. The libraries will then be diluted and denatured according to Illumina's protocol, loading concentrations vary depending on library type. NovaSeq 6000 flowcells are large and require final loading concentrations of ~2nM in 100-310uL, low concentration libraries require larger volume for sequencing on large flow cells. The NovaSeqX flowcells require smaller library volumes. For example, the largest flowcell, 25B, requires a final loading concentration of 2nM in 280uL and the 1.5B flowcell requires 34.2uL. Please see guidelines for pools below. The remaining library volume can be returned to the researcher at the end of the project. We do NOT guarantee cluster yield or quality for libraries prepared outside of the Genome Center, but we are happy to answer any questions you have regarding library preparation.

NOTE: Before making your libraries, please make sure your barcodes are unique and compatible with Illumina Standards.

H. Volume and Concentration Requirements

Please see Table 1 below for the required quantity, volume, concentration, etc. to perform DNA/RNA extraction, library preparation, QC, and sequencing.

Table 1

Sample	Assay	Minimum Quantity*	Minimum Volume	Quality	Minimum Concentration*	Recommended Concentration Range*
Blood	DNA or RNA Extraction	-	3mL, purple top tube	-	-	-
Saliva	DNA or RNA Extraction	-	Oragen kit, as per kit instructions	-	-	-
Fresh/Frozen Tissue	DNA or RNA Extraction	10mg	-	-	-	-
FFPE	DNA or RNA Extraction	10 slides, 3 scrolls	-	-	-	-
Cell Pellets	DNA or RNA Extraction	~100K-1M cells	1 pellet	-	-	-
DNA	Library Prep for WGS/WES	2 µg	20 µL	-	>15 ng/µL	100-200 ng/µL
RNA	Library Prep: Total RNA-Seq	500 ng	20 µL	DV200>30%	>10 ng/µL	100-200 ng/µL
RNA	Library Prep: mRNA-Seq	1 µg	20 µL	RIN>7	>20 ng/µL	100-200 ng/µL
Library**	Sequencing	-	20 µL	-	4nM	>20nM

* Concentrations are listed for fluorescence-based measurements.
** Libraries can vary widely depending on the kit used, see details in descriptions above.
*** Some RNA extracted from FFPE may not meet the above recommended ranges necessary for our standard RNA library prep kits. We do offer additional library kits which have optimization for lower quality FFPE samples. Contact us to discuss at HTGC@pitt.edu

Example Volumes for Library Pools for sequencing on NovaSeq flowcells

		PER LANE	PER FLOWCELL
	Flowcell:	**uL of 4nM library pool**	**uL of 4nM library pool**
NovaSeqX	25B	30	160
NovaSeqX	10B	25	110
NovaSeqX	1.5B	25	50
NovaSeq 6000	S4	NA	300
NovaSeq 6000	S2	NA	120
NovaSeq 6000	SP/S1	NA	100