Sample/Library Submission Guidelines

- Quality starting material is the #1 predictor of success. All samples/libraries submitted will be analyzed and measured for quality and quantity before processing them for sequencing.
- A description of our initial Quality Control (QC) processes and current sample specifications are listed here.

Table 1 – Samples/Libraries Requirements

	DNASeq	RNASeq	Libraries
Volume (µl)	> 45	> 55	See Table 2
Concentration (ng/ µl)	7 - 100	7 - 200	> 5nM (1.5 ng/µl for 450bp)
OD260/OD280	1.8 – 2.2	> 1.7	1.8 – 2.2
OD260/OD230	> 1.5	-	> 1.5
Size (%Fragments > 5K)	> 50	-	Read length + 130bp
RIN/RQN	-	≥ 7	-
Ratio ND/PG	0.2 – 5		
Traces	Distinct HMW peak No RNA	Distinct RNA peak No DNA	Distinct peak at read length + 130bp

Before You Submit Samples or Libraries:

- You must have a recently issued quote (<30 days old) with a unique project number.
- Please reference this project number in all communications.
- Before QC begin, we must have:
  - A valid Purchase Order (PO) number.
  - Samples ID sheet for samples or Barcode Info sheet for libraries.
- Contact us to schedule an in person drop off or shipment time.
- We recommend that you analyze your samples before submitting, to minimize delays and keep your costs in line with the price quoted.
- Check page 6 for shipping and drop-off instructions.
- Samples/libraries are not normally returned so please plan accordingly.

Shipping/Sample Delivery Requirements

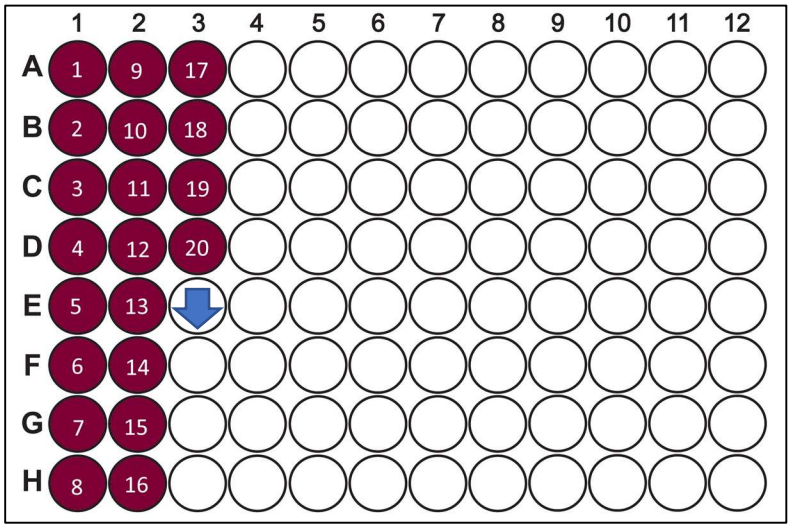
- 96 or 384 well plates are recommended for all studies.
  For example, PerkinElmer Robotic (Cat. No. 6008870) or Bio-Rad PCR (Cat. No. HSS9601) 96 well plates work best.
    - Plates must be sealed with leak-proof strip caps or adhesive seals able to withstand decompression if sent by mail. If you have any doubts about your plate seal, use tubes instead.
    - If stacking more than one plate, please use a separating material like cardboard between plates and use a elastic band to keep them together.
    - Plates should be well-padded to avoid puncture.
- If you do not have access to plates, the next best options is screw top tubes with rubber O-rings. 1.5 ml microcentrifuge or 0.2 ml PCR size tubes.
    - If using non-screw top tubes, wrap lid with parafilm.
    - Send tubes immobilized in a box or rack designed to hold them.

Labeling your Plates/Tubes

- Sample IDs must be provided before work begins. You will receive a Excel-based template file prior to shipping. Sample names must be entered in each well position for each plate.
- Please keep sample names short, simple and easy to read.
- For best results, use letters, numbers, and dashes only. Please no underscore or space.
- Please do not use duplicate sample names. If you need to use the same sample name, add a number to make it unique (such as “Control1” and “Control2”).
- Make sure tubes are clearly and unambiguously labeled. If you are submitting tubes they must be labeled with sequentially numbers on the top and sides.
- Label plates with your Project name and plate number. For example, plate 8 of project 21001Abc should be labeled “21001Abc-08”.

Plate Arrangement

- Plates must be filled by column, as shown in the image below. All our automation assumes column-based ordering.
- If more than 96 samples are submitted, all wells except those in the last plate should be completely filled and the last plate should be filled by column.
- Water or buffer filled "blank" wells will prepped and charged as usual samples.



Quality Requirements

SEE TABLE 1 FOR THE BASIC REQUIREMENTS FOR EACH TYPE OF SAMPLES/LIBRARIES.

DNA (DNA-Seq Preps)

- Genomic DNA should be intact, high molecular weight (HMW), RNase-treated and free of contaminants.
- Sample should be resuspended in EB (10mM Tris in molecular-grade H2O pH 8). Molecular-grade H2O is also acceptable. Do not use buffers that contain EDTA because it has a detrimental effect on the enzyme used in our library prep process.

RNA (RNA-Seq Preps)

- Total RNA should be DNase-treated and dissolved in nuclease-free water.
- RNA samples with significant DNA contamination will be returned and you will be charged for QC.

Libraries for Sequencing

- For client supplied libraries, we are not responsible for any run failures other than those caused by sequencing system or reagent issues. Illumina will determine cause of failure and determine what if any solution we can offer.
- Libraries must be suspended in EB (10mM Tris-HCl, pH 8.0) or RB (10mM Tris-HCl, 0.1% Tween, pH 8.0).
- If your libraries require custom sequencing and/or indexing primers, you must supply them when submitting the libraries. Please let us know ahead of time.
- Follow the Table 2, below, for volume requirements

Table 2 - Volume requirements per platform

Platform	1 Run	2 Runs
MiSeq, iSeq, NovaSeq lane	35 ul	40 ul
NovaSeq SP, S1, or S2 flowcell	55 ul	85 ul
NovaSeq S4 flowcell	90 ul	150 ul

Libraries for Quality Testing Only

- Concentration should be between 1 ng/ul and 3 ng/ul and Volume should be 13ul.
- Needs to be suspended in EB or RSB.
- Unused material will not be returned or used for another project.

DNA/RNA Samples for Quality Testing Only

- Volume should be 10 µl.
- Unused material will not be returned or used for another project.
- Our limit of detection is 5ng/ul for DropQuant and 0.5ng/ul for PicoGreen.

Poly(A) selected or ribosomal-depletion RNA

- Required 1.5ng in 20ul.
- PI shall run QC on the samples prior to treatment.
- No QC will be performed by Genomics and Bioinformatics Service.
- All samples will be prepped with no assurance of outcome.

SARS-COV-2 (Covid-Seq Preps)

- Ct values should be between 12 and 28.
- Volume should be 10 µl.

Initial Quality Control Process

Sample quality control (QC) starts by visually inspecting samples for contaminants and volume discrepancies. "Sample_ID Submission" sheet is reviewed to ensure the number of samples matches the quote, sample volume and concentration, all empty wells are accounted for, and no Sample IDs are used twice.
11 samples are randomly selected per plate for QC, unless the quote states otherwise. Samples selected for QC are analyzed with a fluorometer for concentration estimates and a spectrophotometer for OD ratios. If these samples meet specifications, samples are assayed for integrity. From these measurements, percent of the sample that is less than 5 kb is estimated.

All QC sample data is analyzed, and a Pass/Fail decision is made for each plate:

- Each test sample is scored as "Pass" or "Fail" for each measurement.
- Every test sample with one or more Fail flags is considered a Failed Sample.
- Every plate with two or more "Failed" test samples is considered a Failed Plate.

If a plate fails QC there are commonly three options:

- Plate replacement.
- Possible Corrective actions.
- Or continuing without remediation.

Shipping and Drop-off

- For all communication, please use this email TxGen@ag.tamu.edu and cc Dr. Charlie Johnson at Charlie@ag.tamu.edu
- Drop off samples/libraries between 9:30 and 4:00 Monday through Friday.
- Shipped samples/libraries must arrive during normal buiness house Monday through Friday. Best days to ship Monday through Wednesday for next day arrival.
    - Please forward the tracking number as soon as package ships.
- Shipped samples must remain frozen so pack enough dry ice to last several days.
- Please remember that all submissions must include project name and a signed copy of the quote.
- Shipping/Drop-off address:

    Charlie Johnson
    Genomics & Bioinformatics Service
    1500 Research Parkway, Suite 250
    College Station, TX 77845
    Phone: +1 979 862-3262

- Map of our location here: https://www.txgen.tamu.edu/contacts/location/
- You can find additional shipping information here: https://www.txgen.tamu.edu/contacts