Submission Guidelines

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

Table 1 – Samples and Pools of Libraries Requirements

	DNA	RNA	Pools of 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 or RQN	-	≥ 7	-
Traces	Distinct 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 PO (Purchase Order).
  - 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 additional costs.
- Check page 7 for shipping and drop-off instructions.
- Samples and pools of libraries are not normally returned so please plan accordingly.

Preparing your Plates/Tubes

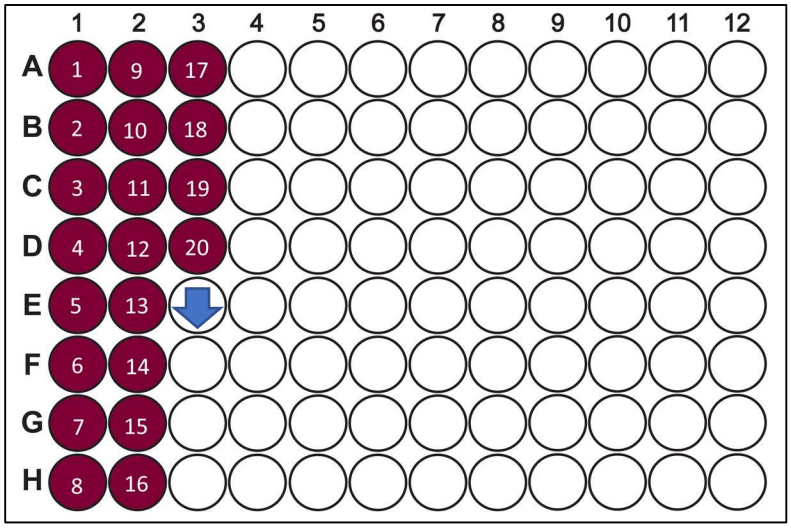
- For DNA/RNA projects with >16 samples use 96 well plates.
    - Use preferably PerkinElmer Robotic (Cat. No. 6008870) or Bio-Rad PCR (Cat. No. HSS9601) 96 well plates.
    - If you don't have plates, contact us and we can provide them at a nominal shipping cost if they need to be shipped.
    - Plates must be sealed with adhesive seals able to withstand decompression if sent by mail.
    - If stacking more than one plate, please use a separating material like cardboard between plates and use a rubber band to keep them together.
- For DNA/RNA projects with ≤16 samples or pools of libraries you can use 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.
- Keep sample names short, simple and easy to read.
- Only use letters, numbers, and dashes. Do not use underscores or spaces.
- 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").
- If you are submitting tubes make sure tubes are clearly and unambiguously labeled. They must be labeled with sequential 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 P08".

Plate Arrangement

- Samples must be ordered by columns, 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 be prepped and charged as usual samples.



Quality Requirements

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

DNA samples for Library Preparation and Sequencing

- Genomic DNA should have more than 50% of fragments larger than 5Kb, be 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 samples For Library Preparation and Sequencing

- 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.

Pools of Libraries for Sequencing

- For client supplied libraries, we are not responsible for any run failures other than those caused by sequencing system issues. Illumina will determine the cause of failure and what solutions we can offer.
- Libraries must be suspended in EB (10mM Tris-HCl, 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 in writing.
- Follow Table 2, below, for volume requirements.

Table 2 - Volume requirements per platform

NovaSeq X Plus		
Flowcell / Lane	**Requirement**	**Extra**
1 Lane - 1.5B, 10B, 25B	37 ul	+12 ul p/lane
1 flowcell - 1.5B	41 ul	+ 16 ul p/flowcell
1 flowcell - 10B	89 ul	+ 64 ul p/flowcell
1 flowcell - 25B	121 ul	+ 96 ul p/flowccell
MiSeq and iSeq		
Flowcell / Lane	**Requirement**	**Extra**
1 flowcell – MiSeq or iSeq	31 ul	+6 ul p/flowcell
NovaSeq 6000		
Flowcell / Lane	**Requirement**	**Extra**
1 lane - SP, S1, S2, S4 XP mode	31 ul	+6 ul p/lane
1 flowcell - SP, S1, S2	55 ul	+30 ul p/flowcell
1 flowcell - S4	87 ul	+62 ul p/flowcell

Pools of 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 (10mM Tris-HCl, pH 8.0).
- Unused material will not be returned or used for another project.

DNA or 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.

Initial Quality Control Process

Sample 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.

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.
- Plate remediation.
- Or continuing without remediation.

Shipping and Drop-off

- Shipping/Drop-off address:

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

- For all communication, please use this email TxGen@ag.tamu.edu and cc Dr. Charlie Johnson at Charlie@ag.tamu.edu
- Please remember that all submissions must include the project name and a signed copy of the quote.

Shipping

- Shipped samples/libraries must arrive during normal business hours Monday through Friday. Best days to ship are Monday through Wednesday for next day arrival.
- Please forward the tracking number as soon as the package ships.
- Shipped samples must remain frozen so pack enough dry ice to last several days.
- You can find additional shipping information here: https://www.txgen.tamu.edu/contacts

Drop-off

- Drop off samples/libraries between 9:30 and 4:00 Monday through Friday.
- Map of our location here: https://www.txgen.tamu.edu/contacts/location/