Sample Submission Guidelines
Genomics and Bioinformatics Service

Quality starting material is paramount to a successful sequencing project. Samples submitted to our facility will be analyzed and measured for quality and quantity before processing them for sequencing. If one or more of your samples fail our quality control (QC) analyses, we will contact you to describe the problem and discuss possible solutions. Most often, the best solution will be to resubmit higher quality samples, which can cause significant delays. Initial QC charges are included in our library prep costs, but you will be charged for additional QC assessments of replacement samples. Therefore, **we ask that you analyze your samples before submitting them to minimize delays and keep your costs in line with the price you were quoted**. *Know what you are submitting before you send it*.

Contacts:

- Dr. Charlie Johnson (*charlie@ag.tamu.edu*) - starting a project, study design, collaborations
- Dr. Richard P. Metz (*rmetz@tamu.edu*) - samples, submission process
- Dr. Marcel Brun (*marcel.brun@ag.tamu.edu*) - data, processing, bioinformatics

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DO NOT SEND OR BRING SAMPLES TO OUR FACILITY UNLESS YOU HAVE A SIGNED QUOTE/SCOPE-OF-WORK AND HAVE DISCUSSED SAMPLE DELIVERY

Before You Submit Samples:

1. You must have a recently issued quote with a unique project name from Dr. Johnson (*charlie@ag.tamu.edu)* before submitting samples to our facility. All communications regarding the project will reference this name. **Please save this information**.
2. Contact Dr. Metz (*rmetz@tamu.edu*) to receive **submission guidelines**, schedule a sample drop off time and to discuss your samples. We will ask you to send quantification data (nanodrop, Qubit, picogreen, etc), and images (agarose gel images, Bioanalyzer, Tape Station or fragment analyzer traces). We can often spot quality issues ahead of time and save everyone a lot of time and money.
3. Prior to sample submission, you will receive an excel-based spreadsheet to communicate your sample IDs and other information. Enter samples ID for each sample where indicated and any other requested information. Please keep a copy of this sheet for your records. **We will not begin work on your samples until we have this information**.
4. Once a sample is submitted for processing, any remaining material will **not be returned**. Investigators should retain a portion of each sample for follow-up validation studies.

LET US KNOW WHAT YOU ARE SUBMITTING BEFORE YOU SEND IT

Shipping/Sample Delivery Requirements

- **For larger projects** (>24 total samples), we ask that samples be delivered in 96-well plates such as PerkinElmer Robotic Plates (Cat. No. 6008870) or Bio-Rad PCR plates (Cat. No. HSS9601). *If you are sending the samples by plane, the plate must be sealed with strip caps or leak-proof adhesive that will withstand decompression in a plane's cargo hold.* If you have any doubts about your plate seal, use tubes instead.
- **For smaller projects** (<24 samples), or if you cannot use plates, our preference for tube types is screw top tubes with rubber O-rings >> 1.5 ml microcentrifuge tubes >> 0.2 ml PCR tubes.
- If you are using tubes, wrap each one with parafilm.
- Send tubes immobilized in a box or rack designed to hold them.
- Unless instructed otherwise, **all samples must be the same volume and concentration**. If this is not possible, you must discuss this with us first.
- **Shipped samples must remain frozen during shipment**. Plates must be well-padded to avoid puncture and the box must contain enough dry ice to last several days.
- If you cannot ship on dry ice and/or in 96 well plates, we can discuss alternatives.

SHIPPED SAMPLES MUST REMAIN FROZEN DURING SHIPMENT

Labeling your Plates/Tubes

- **Sample IDs must be provided before work begins**. Clients will receive a blank Excel-based plate template file prior to shipping. Sample names must be entered in each well position for each plate.
- Please keep sample names simple and easy to read.
- Sample names must not have special characters**.** For best results, use letters and numbers only. If you need to use dashes or spaces, use underscore (_) instead.
- 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 "Control_1" and "Control_2").
- Make sure tubes and/or plates are clearly and unambiguously labeled. If you are submitting tubes that are already labeled, sequentially number them with a different color Sharpie.
- Label plates with your Project name and plate number. For example, plate 8 of project 18001Abc should be labeled "18001Abc_08".

Sample IDs must be provided before work BEGINS

Submission Guidelines by Sample/Prep Type

RNA (For all RNA-Seq and small RNA-Seq Preps and PacBio IsoSeq* preps)

We suggest using column-based kits such as those made by Qiagen and Ambion. If you are using a phenol-based purification system (such as Trizol), further purify the RNA using a column based cleanup procedure to ensure contaminating RNases and phenols are removed. **Isolating small RNA requires kits specifically designed (e.g. mirVana) to retain low molecular weight RNA.**

- Total RNA should be DNase-treated and dissolved in nuclease-free water.
- The OD260/OD280 ratio should be close to 2.0.
- Unless you have discussed it with us before hand, we ask for at least 1000-2000 ng of total RNA in a volume no greater than 50µl.
  ***For PacBio IsoSeq, RNA needs to be as concentrated as possible**.

DNA (DNA-Seq for resequencing, whole genome shotgun, de novo and BACs)

DNA isolation method may depend on desired outcome, sample number and cost. The best DNA comes from CTAB and phenol-based methods, but you may not need it to be so pure. Contact us to discuss DNA isolation methods if you are not sure.

- Genomic DNA should be treated with RNase and dissolved in EB (10nM Tris-HCl, pH 7.5-8.0), TE (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) or nuclease-free water.
- The OD260/OD280 ratio should be 1.6 or higher.
- We require at least 500-2000 ng of high molecular weight, intact DNA in a volume of 50-100µl.

High Throughput, Reduced Representation DNA Sequencing (AgSeq)

The larger number of samples makes column-based, high throughput DNA isolation methods more appealing. Contact us if you need help selecting a method for DNA isolation.

- DNA should be high quality. Best results are obtained from intact, high molecular weight, RNase-treated DNA that is free of contaminants.
- All samples must be the same volume and concentration. The total amount of each sample submitted must be at least 300 ng and no more than 3,000 ng in a volume of 60 µl (5-50 ng/µl) as determined by fluorometric measurement (Picogreen, Qubit, etc.). Ideally, samples should be supplied as 60 µl each with a concentration of 25ng/µl.
- Sample should be resuspended in EB (10mM Tris in molecular-grade H_2O pH 8). Molecular-grade H_2O is also acceptable. Try to avoid TE.
- Samples should be shipped in hard-shell 96-well skirted PCR plates (such as Perkin Elmer Cat. No. 6008870 or Bio-Rad Cat. No. hsp9601) with a non-leaking tight seal or strip caps.
- Plates must be loaded in such a way as to minimize blank wells between samples.
- Plates must be numbered and clearly labeled with smear-proof marker.

gDNA for Long Read Sequencing (PacBio/Oxford Nanopore)

Longer read technologies require intact, high molecular weight DNA and PacBio DNA sequencing requires the purest DNA possible. The prep, and even sequencing itself, is significantly affected by poor quality DNA. Current methods require much more DNA than Illumina-based methods.

- DNA must be free of RNA and dissolved in EB (10nM Tris-HCl, pH 7.5-8.0), TE or nuclease-free water.
- The OD260/OD280 ratio must be 1.6 or higher.
- The OD260/OD230 ratio must be 1.6 or higher.
- Depending on the number of SMRT cells requested, we require at least 3-5 µg of high molecular weight, intact DNA in a reasonable volume.

Amplicons for Illumina-based sequencing (Custom, 16s metagenomics, etc)

We require that you contact us before planning an amplicon-sequencing project. There are many variables at play in amplicon sequencing and we need to make sure we are all on the same page. As of 2019, we require that samples submitted for amplicon sequencing already have Illumina adaptors. We can provide you with tools to help design your primers.

- Amplicons must have Illumina-based adapter sequences already incorporated at both ends.
- Amplicons must be purified (no PCR reactions).
- Must provide an image of the PCR products (gel, Bioanalyzer, etc).
- Must submit at least 50 ng in 50 µl of EB, TE or water.

Libraries

If you are submitting pre-made libraries, you must talk with us first. Depending on the platform, there are vastly different requirements for submission. Final libraries should be in EB (10mM Tris-HCl, pH 8.0) or RB (10mM Tris-HCl, 0.1% Tween, pH 8.0).

- For iSeq, MiSeq and HiSeq: at least 20µl of 10 ng/µl or higher.
- For NovaSeq: At least 200 µl of 5 ng/µl or higher.

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Pricing, FAQs, tips and much more information can be found on our website www.txgen.tamu.edu

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