NCSU Genomics Sciences Lab

High Throughput Sequencing Submission Sheet

Customer Information:

PI Name:_____ Institution: _____

Contact: _____ Email: _____

PLEASE:

- **Make Sure Tubes are Clearly Labeled With PI Name and Sample Name**
- **Please send a hardcopy submission form with the samples when shipping**
- **Attach SAMPLE NAMES and CONCENTRATIONS on a separate sheet**

Libraries:

□ **Prepared Library (please submit 25ul at 1-2ng/ul)**

□ **Library Prep**

□ gDNA □ RNAseq (mRNA) □ small RNA □ Mate Pair

Source Organism: _____

Concentration/Quality Requirements

Concentration: _____ Measured by: (must provide documentation)

□ Gel Estimate □ PicoGreen □ Bioanalyzer □ other_____

For DNA samples, customers must provide a gel image.

Sequencing:

□ **Ion Proton** □ 200 SE (P1 Chip)

□ **Illumina MiSeq** (other run parameters available on request)

v2 chemistries (~15 Million Reads Per Flow Cell)
□ 150x2 PE □ 250x2 PE □ 300SE

v3 chemistries (~25 Million Reads Per Flow Cell)
□ 75x2 PE □ 150 SE □ 300x2 PE

□ **Illumina HiSeq** □ 100x2 PE □ 100 SR
(other run parameters available on request)

□ **PacBio *** □ 10kb lib / P4-C2 SMRT Cell □ 20kb lib / P5-C3 SMRT Cell
(Please see next page for additional notes about PacBio gDNA submission)

Number of Lanes, Chips, or Flow Cells: _____

[Core Facility Use]:

Date Received: _____ By:_____

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If shipping, please email a copy of the sample submission sheet to GSLNCSU@gmail.com so we can prepare and watch for your package.

Genomic DNA Requirements for Illumina or Ion Torrent

- 2µg of genomic DNA in 120µl of TE. (If you are unable to produce this much DNA, please contact the GSL.)
- DNA should be of good quality, with no degradation visible when run on a gel. If possible, DNA should be run on an Agilent bioanalyzer 2100
- OD 260/280 ratio should be between 1.8 and 2
- You must provide a gel image of gDNA with a ladder of a known concentration.

mRNA Sequencing Requirements

- 2µg of total RNA. (If you are unable to produce this much RNA, please contact the GSL.)
- Illumina Samples should be in 50µl of nuclease free water.
- Ion Proton Samples should be in 20ul of nuclease free water
- RNA Integrity Number (RIN) needs to be greater than 7 and samples should have a 28S/18S ratio of greater than 1.6. The 260/280 ratio should be 1.8-2.0 and the 260/230 ratio should be close to 1.8.
- If you have a gel image or BioAnalyzer image, please submit a copy with your sample.

Multiplexing

- The Illumina multiplexing kit offers 24 unique tags that can all be sequenced together in a lane.
- The Ion Proton multiplexing kit offers 16 unique tags that can all be sequenced together on a chip.
- PacBio does not offer multiplexing at this time.

Sample and Library Storage

- The GSL stores all remaining library and starting material at -20 degrees (-80 for RNA) for a period of 90 days after full project completion.
- After 90 days the samples will be disposed of accordingly.
- Users are welcome to pick up samples at any time before this point.

Data Delivery

Untrimmed and un-filtered FASTQ files can be delivered to the customer via:

- A user provided hard drive that will be reformatted at the GSL before use. Label drive with PI name and phone number.
- Over a secure internet connection

Due to space constraints, the GSL is unable to store data.

The user will be notified when sequences are ready for delivery and the GSL will delete files 90 days after sequencing project completion; the user will not be notified before deletion.

***Sample requirements for PacBio sequencing**

PacBio requires 10-20ug of gDNA for library prep. Please contact the GSL for more information.

The Pacific Biosciences library preparation process does not utilize amplification techniques and resulting library molecules are directly used as templates for the sequencing process. As such, the quality of the DNA starting material will be directly reflected in the sequencing results. Any irreversible DNA damage present in the input material (e.g., interstrand crosslinks, etc.) will result in impaired performance in the system. High-quality, high-molecular-weight genomic DNA is imperative for obtaining long read lengths and optimal sequencing performance.

Important measures impacting DNA quality

To maximize read length and quality, it is essential that your DNA sample:

- Is double-stranded; single-stranded DNA is not compatible with the library preparation process.
- Has not undergone multiple freeze-thaw cycles as they can lead to DNA damage.
- Has not been exposed to high temperatures (e.g.: > 65oC for 1 hour can cause a detectable decrease in sequence quality), pH extremes (< 6 or > 9).
- Has an OD/ODratio of 1.8 to 2.0.
- Does not contain insoluble material.
- Does not contain RNA contamination.
- Has not been exposed to intercalating fluorescent dyes or ultraviolet radiation. SYBR dyes are not DNA damaging, but do avoid ethidium bromide.
- Does not contain denaturants (e.g., guanidinium salts or phenol) or detergents (e.g., SDS or Triton-X100).
- Does not contain carryover contamination from the original organism/tissue (e.g., heme, humic acid,
  polyphenols, etc.)

Guidelines for DNA extraction to maintain high molecular weight and clean DNA

These are general recommendations to help maintain high molecular weight DNA.

1. Before DNA extraction:
    1. Avoid incubation in complex or rich media
    2. Harvesting from several cultures rather than a single, high-density culture during early- to mid- logarithmic growth phase is preferred.
    3. Extraction of small volumes is preferred over large volumes to avoid accumulating high concentrations of potentially inhibiting secondary components.
2. Options for DNA Extraction: (no official endorsement from PacBio)
    a. Qiagen® MagAttract® HMW lit (100-200 kb)
    b. Qiagen Genomic-tip kit (50-100 kb)
    c. Qiagen Gentra® Puregene® kit (100-200 kb)
    d. Phenol-chloroform extraction
        i. Ensure phenol is fresh and not oxidized; use within three months of opening of reagent bottle.