Preparation and Shipment of Samples to Maryland Genomics

I. Table of Contents

II. Principle and Scope

a. This document provides the requirements and instructions for shipping samples to Maryland Genomics.
b. The document will provide instructions on how to prepare and check the quality of DNA, RNA, and libraries for sequencing.
c. Clients preparing and shipping their samples to Maryland Genomics are responsible for compliance with this SOP. Samples failing to meet these requirements may be rejected.

III. Specimen Submission Requirements

a. **Sample Tubes and Plates**
   i. Please use properly sealed and labeled 1.5 or 2.0ml tubes or fully skirted or semi-skirted plates. Please reference the appropriate tables below for volume requirements.
   ii. **Do not submit samples in PCR or 0.7 ml tubes**. All samples are tracked with barcodes, and the barcode labels do not fit onto these tubes.
   iii. 1.0 ml Micronic or Matrix tubes with a 2D barcode label on the bottom are preferred. Please indicate the barcode of the tube each sample is in (or the position if sending the tubes in a 96-position rack) using the 'Tube Label' field on the work order form.
   iv. DNA for 16S or other amplicon sequencing should be provided in either **96-well full-skirted plates** or 1.5 - 2.0ml microcentrifuge tubes. See section VI.
   v. For samples sent for nucleic acid extraction, please follow the input guidelines in section IX.

b. **Libraries**
   i. When submitting a sample that will be made into multiple libraries (whether for the same sequencing platform or different platforms), it is preferable to receive the material for all libraries in a single tube.

ii. Please avoid sending the same sample in multiple tubes, unless instructed otherwise.

IV. Illumina Sequencing Requirements

a. Illumina DNA Requirements

i. Samples must meet either the minimum concentration **or** minimum volume requirement.

Library Type	Minimum Amount Required	Minimum Concentration	Minimum Volume
Genomic Paired End	50 ng	10 ng/µl	30 µl
Low Input Genomic Paired End	10 ng	5 ng/µl	30 µl
Exome or Custom Capture	500 ng	20 ng/µl	30 µl
ChIP-Seq	1-2 ng	2 ng/µl	25 µl

ii. **DNA extraction guidelines**

1. Most DNA isolation methods and kits yield material that is suitable for Illumina sequencing. Treating DNA samples with RNase is recommended.
2. If a CTAB (Cetyl trimethylammonium bromide) protocol is used to purify DNA, it should be followed with an ethanol precipitation or column-based purification to remove impurities introduced during the extraction.
3. For additional extraction guidelines, please refer to section X.

b. Illumina RNA Requirements

i. Samples must meet either the minimum concentration **or** minimum volume requirement.

Library Type	Minimum Amount Required	Minimum Concentration	Minimum Volume
RNAseq (with poly-A selection) from total RNA	100 ng	20 ng/µl	30 µl
Low-input RNAseq (with poly-A selection) from total RNA	10 ng	2 ng/µl	10 µl
RNAseq (with ribo-reduction) from total RNA	100 ng	10 ng/µl	30 µl
RNAseq from mRNA	50 ng (100 ng preferred)	5 ng/µl	20 µl
Small RNA	25 ng of total RNA	300 ng/µl	10 µl

ii. **RNA Extraction Guidelines**
1. Samples for small RNA sequencing and those that will not undergo poly-A selection must not be in TE.
2. For additional extraction guidelines, please refer to section X.

c. **Illumina Prepared Library Requirements**

Library Type	Minimum Amount Required	Minimum Concentration	Minimum Volume
Individual Prep	60 nano moles	2nM	30 µl
Pooled Samples	60 nano moles	2nM	30 µl

V. PacBio Sequencing Requirements

a. **PacBio DNA Requirements**

Library Type	Prep Type	Size Selection	Minimum Amount Required	Minimum Concentration	Minimum Volume
HiFi (CCS)	Multiplexing/Amplicon	Yes, for 2-3 plex	2 µg	25 ng/µl	30 µl
		Yes, for 4-96 plex	50 ng - 1ug	25 ng/µl	30 µl
	Standard	Yes	5 µg - per 1 Revio cell	25 ng/µl	30 µl
	Low Input	No	1 µg	10 ng/µl	30 µl
	Ultra-low	Yes	5 ng	1 ng/µl	10 µl

i. Submitted samples must meet either Minimum Concentration or Minimum Volume requirement. Ensure submitted samples are free of proteins, polysaccharides, and detergents. Check sample quality on Nanodrop. **Preferred ratios are: 1.8+/- 0.1 for 260/280, 2.0 – 2.2 for 260/230**. We recommend against sequencing samples extracted from agarose gel. Agarose contamination may inhibit SMRT Cell loading and current QC methods DO NOT detect this prior to loading.
ii. Our standard HiFi library size is 15-20kb based on PacBio recommendation. But we can accommodate sizes outside of this range upon request. DNA samples intended for HiFi library should have more than 50% of the input DNA larger than 30kb.
iii. For Ultra-low input HiFi prep, library will be PCR-amplified and size-selected on Blue Pippin.
iv. DNA samples for PacBio sequencing should be suspended in 10mM Tris-HCl (PH8.0 or higher) or Low 1XTE with 0.1mM EDTA.

v. If DNA has come in contact with phenol, CTAB or other detergents, or may contain salts or other contaminants, the sample should be purified by ethanol precipitation before being submitted to Maryland Genomics to remove any remaining traces of these contaminants. These contaminants can affect sample quantitation, enzymatic steps, and sequencing, and may not be removed by the purification steps that are a part of the library preparation.

vi. **DNA Extraction Publication Method for PacBio**

1. **Please follow this link for PacBio DNA extraction guidelines:** https://www.pacb.com/wp-content/uploads/Technical-Note-Preparing-DNA-for-PacBio-HiFi-Sequencing-Extraction-and-Quality-Control.pdf
2. For additional extraction guidelines, please refer to section X.
3. **DNA Extraction kits suggested by PacBio:**

Kit	Part number
QIAGEN MagAttract HMW DNA Kit	67563
QIAGEN Genomic-tip 20/G Kit	13343
QIAGEN PAXgene Blood DNA Kit	761133
NEB Monarch Genomic DNA Purification Kit	T3010S
PacBio Nanobind CBB Kit	102-301-900
PacBio PanDNA kit	103-260-000

b. **PacBio RNA Requirements**

i. Samples must meet either the minimum concentration **or** minimum volume requirement.

Library Type	Prep Type	Size Selection	Minimum Amount Required	Minimum Concentration	Minimum Volume
Iso-Seq	Total RNA	No	300 ng	10 ng/µl	30 µl
	cDNA	No	150 ng	5 ng/µl	30 µl
Kinnex	Total RNA	No	300 ng	10 ng/µl	30 µl

c. **PacBio Kinnex Single-Cell Requirements**

i. Samples must meet either the minimum concentration **or** minimum volume requirement.

Library Type	Prep Type	Size Selection	Minimum Amount Required	Minimum Concentration	Minimum Volume

Kinnex	Single Cell cDNA	No	15 ng	5 ng/µl	30 µl

d. **PacBio Prepared Library Requirements**
   i. Please contact us if your multiplexed library pool has an insert size larger than 20kb.
   ii. If a library requires 3 or more SMRT Cells, the required amount must be re-calculated based on QC results.
   iii. Libraries must meet either the minimum concentration **or** minimum volume requirement.

Platform	Library Type	Minimum Amount Required	Minimum Concentration	Minimum Volume
Sequel IIe	Single	80 pico moles	2nM	10 µl
	Multiplexed	100 pico moles	2nM	10 µl
Revio	Any	300 pico moles	2nM	15 µl

VI. Amplicon Sequencing Requirements

a. **DNA requirements**
   i. DNA samples should be quantified with a PicoGreen Assay or the Qubit Assay. Please include final concentrations on the sample submission form**.**
   ii. Send at least 30 µl of DNA per sample.
   iii. Samples will be assessed by digital PCR for target copy number prior to library construction. We will advise on any samples that are unlikely to yield sufficient libraries prior to sequencing.
   iv. Send at least 30 µl of DNA per sample.
   v. Sample well location in the 96-well full-skirted plate should be recorded on the sample submission spreadsheet in column order (i.e: A1, B1, C1…). If sending in tubes, please appropriately label each tube to make it identifiable. Any unknown sample tube will be set aside until confirmed.

b. **16S PacBio Sequencing Sample Requirements**
   i. Samples must meet either the minimum concentration **or** minimum volume requirement.

Library Type	Sample Type	Size Selection	Minimum Amount Required	Minimum Concentration	Minimum Volume
Kinnex	16S rRNA Amplicon	No	35 ng	2 ng/µl	30 µl

VII. NanoString nCounter Requirements

a. **RNA Requirements for NanoString**

i. Samples must meet either the minimum concentration **or** minimum volume requirement.

Run Type	Amount Required	Minimum Concentration	Minimum Volume
Gene Expression	25-100 ng total RNA	20 ng/µl	8 µl
miRNA	100 ng total RNA or 10 ng of miRNA	20 ng/µl	8 µl

VIII. 10X Genomics Single Cell Experiment

a. Cell Preparation

i. 10X Genomics® Single Cell Protocols require a suspension of viable single cells or nuclei as input. Minimizing the presence of cellular aggregates, dead cells, non-cellular nucleic acids, and potential inhibitors of reverse transcription is critical to obtaining high quality data.

ii. For detailed information on best practices for handling cells and preparation for 10X Genomics experiment, please refer to 10X Genomics Single Cell Protocols “Cell Preparation Guide”, which can be found at the links below:

1. **https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep**
2. **https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/single-cell-gene-expression-demonstrated-protocol-compatibility-table**

iii. The ideal concentration for cell suspensions is 700-1200 cells/ul for NextGEM assays and 1300-1600 cells/ul for GEM-X assays.

b. Cell Counter

i. Maryland Genomics uses the Countess III from Invitrogen. The counter assesses cell viability via trypan blue exclusion. The counter will also provide total cell count with or without trypan blue staining. We are not able to count nuclei. Nuclei must be counted and assessed for viability before arrival.

ii. Use the following guidelines to determine whether the counter will effectively assess the samples for the downstream experiment:

1. Cell Concentration Range: 50,000 – 10,000,000 cells/mL
2. Optimal Concentration Range: 100,000 – 5,000,000 cells/mL
3. Cell Diameter Range: 6 – 50 µm
4. Cell counting will require 10 µL of sample
5. Trypan Blue measures cell viability by reacting with the cell’s internal region of damaged cell membranes, staining the

region blue while intact cell membranes remain bright and unstained. If the samples do not contain any cytoplasm for the dye to bind to, the counter will not be able to count the cells. Viability of the cell suspension should be >90%.

c. **Sample Submission**
    i. 10X Genomics recommends that single cell suspensions be counted and used within 30min. of preparation. **Therefore, a two-week notice is required to set a date for the experiment if a 10X work order will be submitted. In addition, samples must be delivered to Maryland Genomics by 2pm on the day of experiment to ensure completion of sample processing within the same day.**

IX. Submission of Specimens for RNA, DNA or HMW DNA Extractions

a. **Collection Types**
    i. **Collected Swabs in Transport Buffer**
        1. Swabs can be left in the transport buffer. **Please indicate the transport buffer as this affects the extraction method to be used.**
    ii. **Low Biomass specimens**
        1. For samples that are expected to have low microbial biomass, please send at least 1g of sample in case of repeat extractions.
    iii. **Tissues and whole specimens**
        1. Collected fresh and then flash frozen to preserve DNA integrity but can be stored in DNA/RNA protect media (or similar) according to manufacturer's instructions. Ethanol preservation is also accepted. Tissues or whole specimens arriving in some form of media should have their storage buffer noted on the submitted Work Order form under the “Buffer” column.
    iv. **Cells**
        1. Collected fresh, pelleted, supernatant removed, and flash-frozen to preserve DNA integrity but can be sorted in DNA/RNA protect media (or similar) according to manufacturer's instructions. Shipment in lysis buffers is also accepted. Cells arriving in some form of media should have their storage buffer noted on the submitted Work Order form under the “Buffer” column.
    v. **Whole Blood**
        1. Collected fresh and either flash frozen or, if flash freezing is not an option, stored in either an EDTA tube or with a DNA/RNA protect media (or similar). Blood arriving in some form of media should have its storage buffer notes on the submitted Work Order form under the “Buffer” column.

b. **Extraction Input Requirements**

i. The input requirements for DNA and RNA extractions vary depending on the kind of specimen submitted. The amount of specimen provided for extraction should be included in the submitted Work Order under the "Mass (mg)" column. For cell pellets, please include the number of cells provided alongside your sample description in the "Description" column.
ii. Below is a table to serve as a general guide for the inputs of a variety of specimen types for a ***single*** extraction attempt. It is highly recommended to discuss the experiment specifics with the Maryland Genomics team prior to shipment to ensure specimens are properly handled for the best chance of a successful extraction.

Specimen Type	Extraction Input Requirements
Mammalian/Amphibian/Reptilian/ Avian/Aquatic Tissues: Muscle, Brain, Heart, Liver, Kidney, Spleen, Gonad	30-50mg
Plant Tissues	100mg
Fish Skeletal Muscle	100mg
Nucleated Whole Blood	20-50 µl
Non-Nucleated Whole Blood	200 µl
Cultured Mammalian Cells	$3x10^6$ to $1x10^7$ live cells for standard RNA and DNA $1x10^6$ to $5x10^6$ live cells for HMW DNA
Gram (-) or (+) Bacteria	$5x10^8$ - $2x10^9$ live cells
Collected Swabs in Transport Buffer	500 µl
Fecal	1g
Environmental (sand, soil, etc)	1g

X. Extraction Guidelines

a. **RNA Extraction for Illumina Sequencing, PacBio Kinnex Sequencing or NanoString Analysis**
   i. Most RNA isolation methods and kits yield material that is suitable for sequencing. Confirm with Maryland Genomics before shipment.
   ii. RNA samples should be suspended in Molecular Biology Grade Water (or similar) or Tris. Samples must be prepared in a way that does not leave residual DNase activity.
   iii. It is recommended that all RNA samples are DNase treated prior to submission except for RNA samples that will undergo poly-A selection during their RNAseq library preparation.
   iv. Please confirm the absence of DNA by PCR.
   v. Maryland Genomics can perform DNase treatment of RNA samples. This must be included in quoted work and in the submitted Work Order form.

b. **HMW Extraction for PacBio Sequencing**
   i. The PacBio system is extremely sensitive to DNA damage, contaminants in the sample, and molecules that might be bound to

the DNA (polysaccharides, proteins, etc). Some types of DNA damage will be repaired as part of the library prep, but care should be taken to prevent damage during the extraction and storage of the samples.

ii. Events that can result in DNA damage should be minimized. Causes of DNA damage include freeze/thaw cycles, exposure to high temperatures (>55°C for an hour), exposure to extreme pH (<6 or >9), exposure to intercalating dyes, exposure to ultraviolet radiation, x-rays and gamma rays, and long-term storage at 4°C.

iii. Contaminants that can affect sequencing success include RNA, biological macromolecules (polysaccharides, lipids and proteins), insoluble organic contaminants or metabolites, divalent metal ions (eg: MG^{2+}), salts, phenols, and detergents (SDS, Triton X-100, and CTAB.

iv. For information on PacBio recommended DNA extraction kits and extraction methods, please refer to section V.

XI. Sample Quantitation

a. **Quantitation of DNA and RNA**

i. The preferred DNA quantitation method is the Thermo Fisher Quant-iT PicoGreen Assay or the Qubit Assay to measure DNA concentration.

ii. A Nanodrop or other spectrophotometer can be used to obtain a preliminary quantitation of the DNA or RNA.

iii. Spectrophotometry typically overestimates the amount of DNA or RNA in a sample, often as much as ten-fold. If this is the only method of quantitation available, please plan to ship more material.

iv. A spectrophotometric concentration much higher than the concentration determined by other methods is indicative of sample contamination (e.g. phenol or salt carryover). If this occurs for samples to be sequenced on the PacBio platform, the details of sample origin and preparation should be examined, and the sample should undergo additional purification prior to being submitted to Maryland Genomics.

v. Gel Electrophoresis

1. Genomic DNA should appear as a high molecular weight band. If DNA is degraded, it will appear as a smear. A small amount of degradation is acceptable for Illumina sequencing. For PacBio sequencing, the DNA should be intact.
2. For large-size genomic DNA, it is recommended to analyze sample integrity on a Femto Pulse or Fragment Analyzer instrument.

b. **RNA Sample Quantitation and Qualitative Analysis**

i. Bioanalyzer (preferred method)

ii. RNA samples gel method (qualitative analysis)

1. The gel image of your RNA sample should reflect the following: High quality RNA will show a 28S rRNA band at

4.5 kb that should be twice the intensity of the 18S rRNA band at 1.9 kb. The mRNA will appear as a smear from 0.5–12 kb.

c. **Library Sample Quantitation and Qualitative Analysis**
   i. All library types can be submitted to Maryland Genomics for sequencing. Please quantify libraries prior to submitting. Library quantification methods:
      1. Bioanalyzer (preferred method for Illumina libraries)
      2. Fragment Analyzer (preferred method for PacBio libraries)
      3. Femto Pulse (preferred method for large-size PacBio library)

d. **Ethanol Precipitation**
   i. **If a sample buffer needs to be exchanged or a sample needs to be concentrated the following procedure can be performed:**
      1. Add 0.1x volume of 3M Sodium Acetate (pH 5.2) to the sample and mix
      2. Add 3.5x volumes of 100% Ethanol to the sample and mix
      3. Incubate at -80° C for at least 30 minutes
      4. Centrifuge at 4° C at maximum speed for 15 minutes
      5. Remove supernatant
      6. Add 500 µl of 70% Ethanol
      7. Centrifuge at 4° C at maximum speed for 15 minutes
      8. Remove supernatant and allow sample to air dry
      9. Elute with 10 µl (for small RNA library samples) or 35 µl of the desired buffer

XII. Shipping Samples to Maryland Genomics

a. **Work Order Submission**
   i. Ensure the appropriate work order spreadsheet is completed and submitted online prior to shipment. Quality analysis files (gel images or bioanalyzer images) can be e-mailed to mdg-workorders@som.umaryland.edu.
   ii. Work orders should be submitted using our online portal: https://marylandgenomics.org/work-order-submission/
   iii. Microbiome Projects
      1. Projects for 16S and other microbiome amplicons should be coordinated with Mike Humphrys and Lisa Bilski: mhumphrys@som.umaryland.edu and lbilski@som.umaryland.edu.

b. **Shipment**
   i. Tube submission:
      1. Please aliquot your sample(s) into labeled 1.5 ml microcentrifuge tube(s) and seal the top with parafilm.
   ii. 96-well plate submission:
      1. If sending DNA, please aliquot at least 30 µl into designated well locations that match the plate submission form.
      2. Submission forms for 16S microbiome projects must be returned to Mike Humphrys and Lisa Bilski:

mhumphrys@som.umaryland.edu and lbilski@som.umaryland.edu.

iii. **Samples must be shipped overnight on dry ice in a Styrofoam container, along with a hard copy of the work order spreadsheet. Samples should not be shipped over the weekend**. Please e-mail mdg-workorders@som.umaryland.edu with the project name, the anticipated arrival date, and the tracking number for the shipment, along with the electronic work order.

iv. Address:

Maryland Genomics at Institute for Genome Sciences
University of Maryland, Baltimore
ATTN: Lisa Sadzewicz ***
670 West Baltimore St, 3rd Floor
Baltimore, MD 21201
410-706-6734

***** for microbiome projects please ATTN: Lisa Bilski**

v. **NOTE**: International shipments typically take an additional 24-48 hours to clear customs.

vi. If you have concerns about the quantity and/or quality of the sample(s), contact Maryland Genomics prior to shipment.