

Sample requirements for Illumina Sequencing

DNA isolation

Ensure that your DNA sample:

- Is double-stranded. Single-stranded DNA will not ligate to adapters in the library preparation process and can interfere with quantitation.
- Has undergone a minimum of freeze-thaw cycles.
- Has not been exposed to high temperatures (> 65°C for 1 hour can cause a detectable decrease in sequence quality), pH extremes (< 6 or > 9).
- Has not been exposed to intercalating fluorescent dyes or ultraviolet radiation. **If purifying DNA from a gel fragment, DO NOT use ethidium bromide for staining. We recommend SYBR Safe DNA Gel Stain with visualization on a blue light box (<https://www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/nucleic-acid-gel-electrophoresis/dna-stains/sybr-safe.html>). UV will damage. Even a few seconds of UV irradiation appears to render DNA non-sequenceable.**
- Has an OD260/280 ratio of approximately 1.8 to 2.0 (Nanodrop). Also, the OD260/230 ratio should be higher than 2.0
- Does not contain insoluble material.
- Does not contain RNA contamination.
- Does not contain chelating agents (i.e. EDTA), divalent metal cations (i.e., Mg²⁺), denaturants (Guanidinium salts, Phenol), or detergents (SDS, Triton-X100).
- Does not contain carryover contamination from the starting organism/tissue (heme, humic acid, polysaccharides, polyphenols, etc.). Some DNA isolation procedures might contain some contaminant(s) that can affect the quality of the sequencing run.
- Is quantified by a fluorometric method (QUBIT or PicoGreen). Nanodrop (absorbance) is not adequate for quantification, although it does provide valuable information about sample's purity.

Requesting library construction services

The NGS core lab offers library construction services on a limited number of applications. Other NGS sample preparation services are offered

in the Gene Expression & Genotyping core. Please see table provided below.

These guidelines apply to all libraries intended for sequencing on any of the Illumina instruments, regardless of the application (e.g., ChIP-seq, ATAC-Seq, WGBS, RRBS, RAD-Seq, GBS, RNA-Seq, etc.).

For Library Construction	Amount required	Input Quality and Amount, Comments
Standard DNA-Seq	0.2-1.0 µg per sample	If DNA is <5kb (e.g., FFPE), call Core lab
PCR-free DNA-Seq	>250 ng	Requires qPCR library quantification
Low-input DNA-Seq	10-50 ng	Sample will be processed using low-input protocol
Ultra-low input DNA-Seq	0.2-5.0 ng	Sample will be processed using ultra-low input protocol
Whole-genome-bisulfite converted (WGBS)	>200 ng	If sample is <5kb or amount is <200ng, call Core lab
Reduced Representation BS (RRBS)	>200 ng	If sample is <5kb or amount is <200ng, call Core lab
16S Metagenomics (V3 and V4)	50 ng per sample	Submit samples to the GeneExpression/GT core
RNA-Seq	>100 ng total RNA of oligo dT capture. >500 ng for ribodepletion	Submit samples to the GeneExpression/GT core (GE/GT) Best if RIN>7. Call GE/GT core if RIN<7, or amount is <100 ng
10X Genomics, Linked-reads	>100 ng	DNA must be >30 kb. Core offers size selection services if desired (will need ~1 µg). 10X Controller runs are done on a minimum of 8 samples.
10X Genomics, ATAC-seq single nuclei	Pre-isolated, single nuclei	DNA must be >30 kb. Core offers size selection services if desired (will need ~1 µg). 10X Controller runs are done on a minimum of 4 samples.
10XGenomics Single Cell 3'/5' RNAseq Library	Please contact GeneExpression Core	Please contact GeneExpression Core
10XGenomics Single Cell Human/Mouse T-cell V(D)J Library	Please contact GeneExpression Core	Please contact GeneExpression Core
10XGenomics Single cell CNV Library	Please contact GeneExpression Core	Please contact GeneExpression Core
10XGenomics Single cell feature barcode for CITE-seq	Please contact GeneExpression Core	Please contact GeneExpression Core
10XGenomics Single CNV Library	Please contact GeneExpression Core	Please contact GeneExpression Core

Takara single-cell library	Please contact GeneExpression Core	Please contact GeneExpression Core
IDT Exome capture library	Please contact GeneExpression Core	Please contact GeneExpression Core
Agilent SureSelect library	Please contact GeneExpression Core	Please contact GeneExpression Core

16S Metagenomics sequencing recommendations

Illumina Metagenomics libraries are constructed in the GeneExpression/Genotyping core. To start your project, please contact ICBR-Genotyping@ad.ufl.edu (or Yanping Zhang: yanp@ufl.edu) for more details and for planning your experiment. See also “FAQ: 16S metagenomics”

Full-length 16S Metagenomics sequencing is also supported on the PacBio SEQUEL. Please contact us at ICBR-NextGen@ad.ufl.edu to discuss your project.

A similar approach is used for any targeted sequencing experiment. The basic steps for the entire process are as follows:

1. **DNA isolation.** The challenge here is to use a method that extract DNA with “similar” efficiency for all organisms in the sample in order to reveal full diversity in the sample. See also DNA isolation notes above.
2. **DNA quantification.** Use QUBIT (fluorescence-based method). Nanodrop (absorbance) is not adequate for quantification, although it does provide valuable information about sample’s purity. Gel electrophoresis (or equivalent... TapeStation) may also be important if you’re concerned about the integrity of the samples.
3. **Library construction** (16S amplicon or any other target). [ICBR offers 16S library construction services through the GeneExpression and Genotyping core lab](#). If you are constructing own libraries, we recommend the method described by Illumina and the barcoding reagents provided in the Nextera XT kit (Illumina Inc.). (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). The method consists of two sequential PCR reactions. You will need to order [HPLC-purified](#) primers and PCR reagents for the first reaction. The first PCR is exactly the same for every sample. In contrast, the second PCR reaction is unique for every sample. That’s because you need a unique barcode for each sample. You can barcode up to 96 samples to be

pooled for a single MiSeq run (2x300 cycles), using the Nextera XT kit. Other library construction protocols are also accepted. These include, for example, libraries constructed with commercial kits (e.g., ZymoResearch, Swift Biosciences, Qiagen, Bioo Scientific, etc.), or custom protocols as described in the literature.

4. **Library quantification, sizing, and pooling.** Use QUBIT for quantification, and the TapeStation (or Bioanalyzer) for sizing. If you have followed the Illumina recommended method for amplifying variable regions 3 and 4, the size of your library should be ~600 bp). Calculate molarity using Qubit concentration and average size on TapeStation, and pool equimolarly.
 5. **Requesting sequencing services.** To use our facility, your lab will need to have a CrossLab account (PI and lab members). From our website (www.biotech.ufl.edu), use your account credentials to login CrossLab and select UF-ICBR core. Navigate to the NextGen Sequencing core> Illumina sequencing services> MiSeq, etc. to fill out service request form.
 - For 16S (variable regions 3 and 4), we recommend MiSeq, version 3, 2x300 cycles with 20% PhiX spike-in and 10 pM loading. For a pool of 96 samples, this run format should yield ~100k reads per sample. Of course, it is your choice to request any other run configuration based on the goals of your experiment. Please keep in mind that version 2 configurations (e.g., 2x250 cycles) generate roughly half the number of reads as version 3 reagents (e.g., 2x300 cycles).
 - Submit pool to the ICBR NextGen DNA sequencing core. We feel that it is best practice to request independent library pool evaluation services before sequencing, including quantification of “functional” library molecules by qPCR. You can choose to decline these services, in which case you will need to provide the core with all QC data for your pool, plus detailed conditions for sequencing run set up on the MiSeq. We need at least 15 ul at 10 nM (~3-5 ng/ul).
 - Data analysis. This step is not included in our sequencing services. You will need to do that on your own using your favorite method, or request data analysis services to ICBR Bioinformatics Core (ICBR-Bioinformatics@ad.ufl.edu).
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16S Metagenomics Custom Libraries

There are numerous protocols in the literature that have been successfully used for 16S (e.g., protocol adopted by the Earth Microbiome consortium, protocols that use so-called heterogeneity adaptors, protocols from commercial providers such as Bioo Scientific, Qiagen and Zymo Research, etc.).

Some of these protocols use custom-designed barcodes that require custom sequencing primers and/or custom indexing primers. ICBR does NOT provide custom primers for sequencing. If you decide to use any of these custom library protocols, you will need to provide the Core lab with at least 10 ul of custom sequencing and/or indexing primer at 100 µM.

Please see also: [NGS Best-practices](#) document for section on considerations when using custom library protocols.

Sample Shipping information

- We typically recommend samples be shipped in dry ice. Microcentrifuge tubes (1.5-ml) should be firmly capped, further secured with saran-wrap or parafilm, and placed inside 50 ml conical tubes for shipment.
- If preferred samples can be lyophilized and sent by "regular" mail. If you need to declare anything, please indicate that they are "non-toxic nucleotides samples".

ICBR CORE FACILITY INFORMATION	
<i>Shipping Address</i>	<i>Request Information: Contact</i>
NextGenSeq Laboratory	Email: ICBR-NextGenseq@ad.ufl.edu
CGRC Room 178, Univ. of Florida	Tel: (352) 273-8050
2033 Mowry Road	Fax: (352) 273-8070
Gainesville, FL 32610	
<i>For 16S Metagenomics library construction services, please contact ICBR-GeneExpression@ad.ufl.edu</i>	
<i>For Data Analysis services, please contact ICBR-Bioinformatics@ad.ufl.edu</i>	