So, you want to sequence…

A beginner's guide to sequencing your nuclei acid samples at the Genomics Technology Core at the University of Missouri

Introduction.

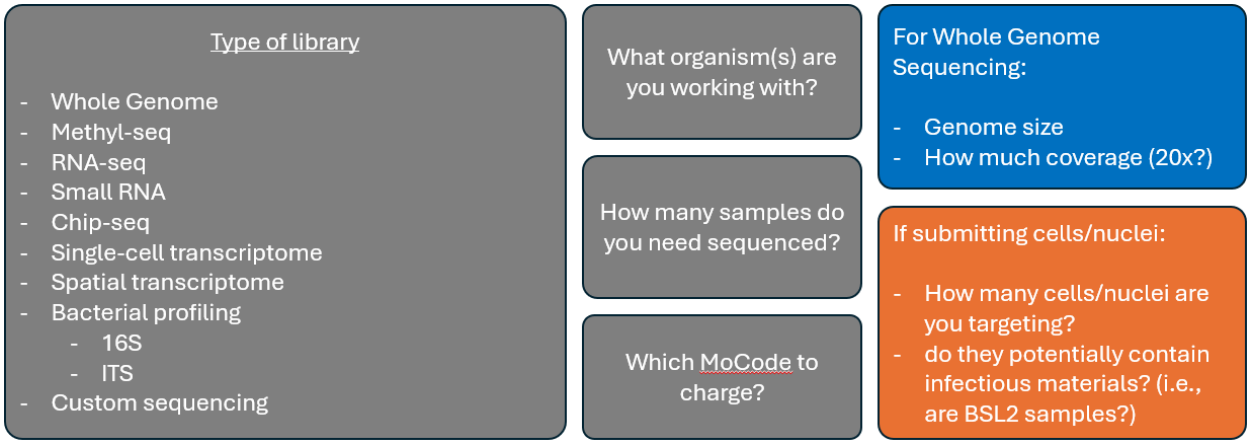
Next generation sequencing (NGS) is a constantly evolving technology that must be used to stay competitive in the current research environment. We at the Genomics Technology Core (GTC) are here to help with your sequencing needs to advance your research (and secure that next research grant). There are many things you may want to sequence: **whole genomes** (DNA library), **methylome** (methyl-seq), **bulk transcriptome** (RNA-seq), **single-cell transcriptome**, **spatial transcriptome**, **small RNA**, **chromatin-immunoprecipitated** DNA (ChIP-seq), **researcher-prepared libraries**, **environmental/biome samples** (for 16S or ITS profiling), or even a **PCR product/plasmid** (Sanger sequencing).

There are many aspects to NGS that can be confusing and so have written this guide to help walk you through the process of submitting samples for sequencing. Each sequencing type requires a certain amount of quality control checks that must be done by the lab submitting the sample (the "researcher") so we can efficiently provide you with the best possible sequence data. Throughout this guide, the main GTC webpage will be linked to. In addition to these links, this guide provides checklists for each sample type to help collect the information needed to submit your sample(s).

Initiating a quote.

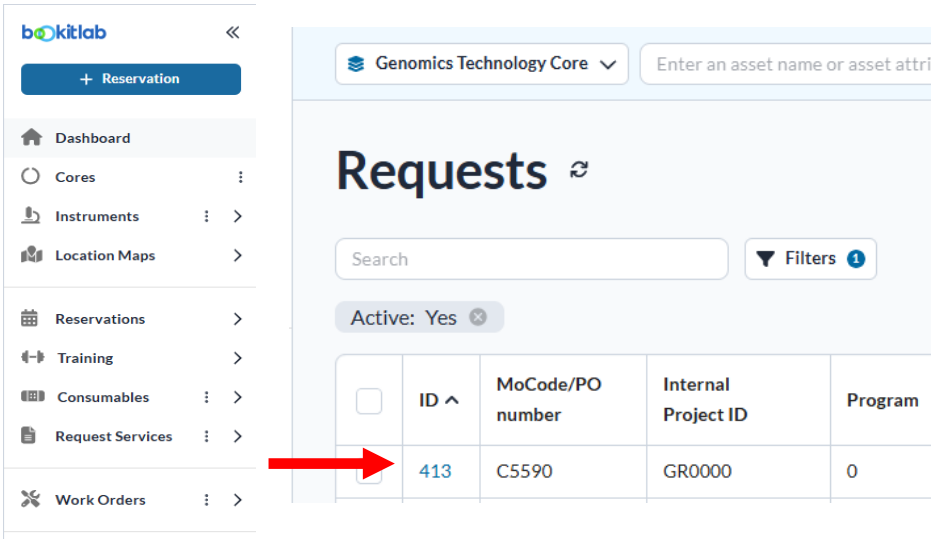
All projects (i.e., anything other than Sanger sequencing samples) should first be discussed with the GTC core director (Nathan Bivens: bivensn@missouri.edu) so that a **Quote** can be generated.

Details needed to initiate a quote:

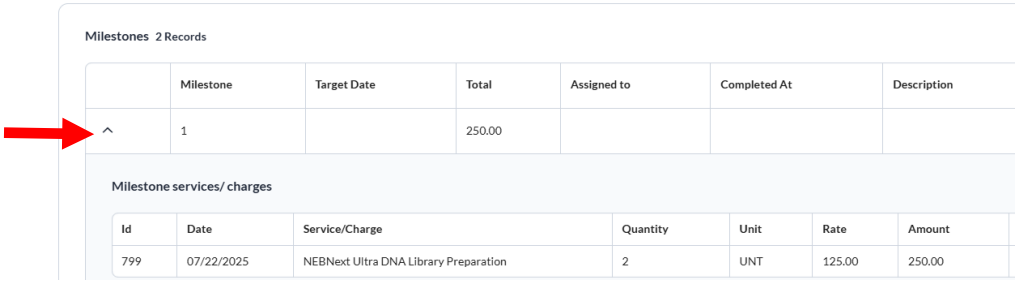


Approving Library Preparation and Sequencing costs.

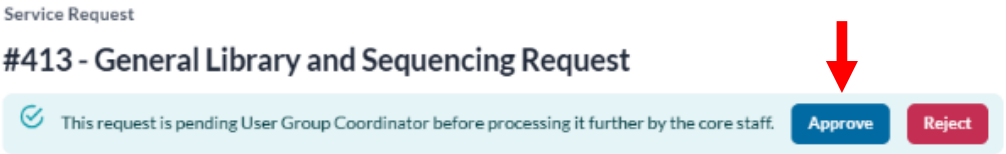
Our billing services are run through Bookitlab, a web-based billing portal. After initiating a project, it will need to be approved through Bookitlab by the head of the lab (usually the PI). An email with a log-in link to Book-it Lab will be provided when the project is initiated. Upon logging into Bookitlab, click on the Requested Services menu along the left side of the screen and click the ID number for the project to be approved.



A list of quoted charges can be found within the Milestone section of the quote. Clicking on the arrow buttons on the left side will expand the section with additional charge details. Note: typically, the library and QC charges will be listed within Milestone 1 and sequencing charges will be listed within Milestone 2.



After confirming the charges are as expected/discussed, click the approve button at the top of the page.

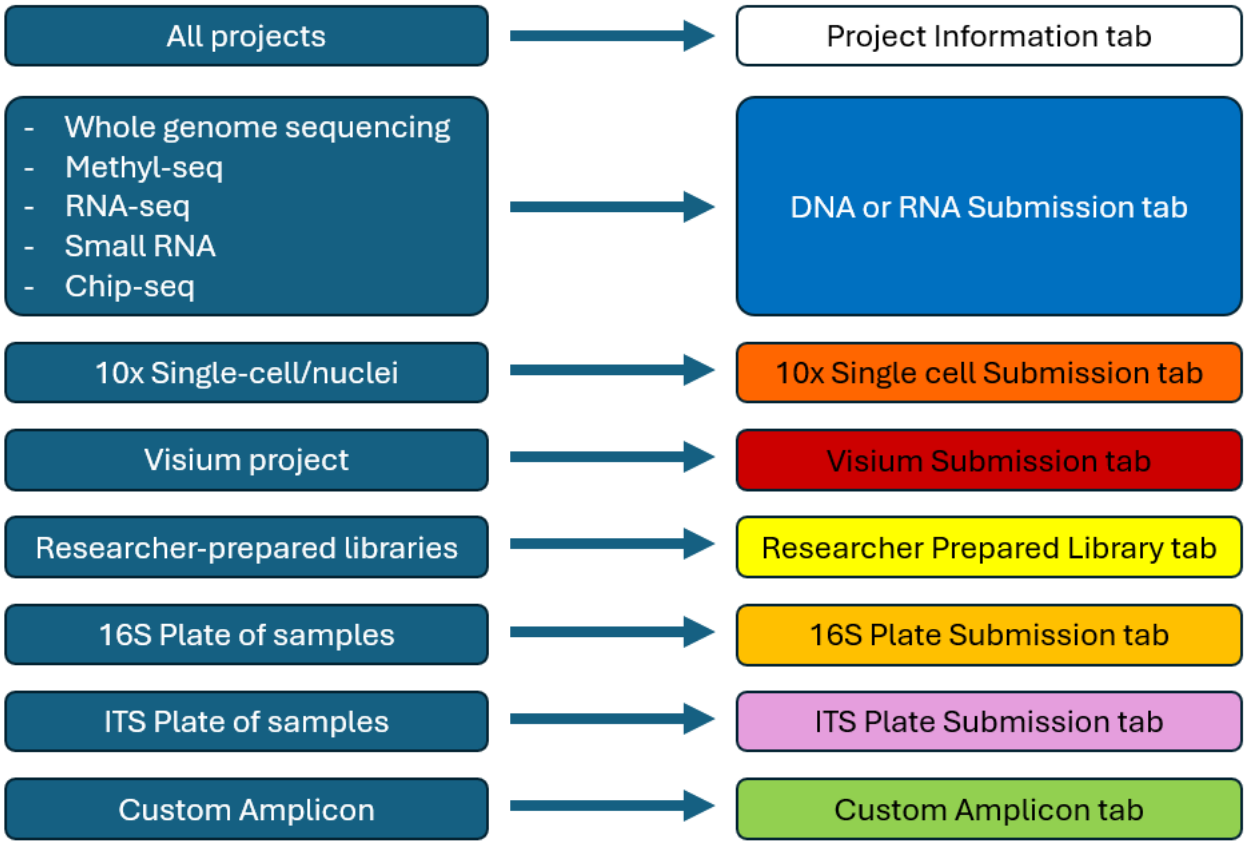


Within a few minutes an email will be sent to confirm the project is approved and the status will now show as “InProcess”.

Alternatively, a project can be approved by clicking the “Approve Request” link in an email sent by Bookitlab. With this approval process, no confirmation email is sent but approval can be confirmed by viewing the update to the project status on the request webpage. Click the “More/Edit” link at the top of the request page and note the change in status as “InProcess”.

Necessary paperwork.

Prior to submitting the samples, we request that the researcher fill out and return the supplied Excel file: **Project Information Form.** This form is supplied to the researcher(s) by email. There are several tabs on the Project Information Form that should be considered:



The GTC director will assign your project a **Project ID**, which can be found in the quote. The Project ID will have the form GR####, with a unique number to distinguish it from other projects. When the samples are brought to the GTC, please make sure to inform the personnel which Project ID the samples being submitted are associated with so that the samples can be appropriately stored and tracked.

Submitting samples.

Since library construction requires the use of many magnesium-requiring enzymes, all samples should be free of metal chelating compounds (e.g., EDTA). We request these not be present in the resuspension/elution of samples. (Tip: the E in TE stands for EDTA.)

DNA and RNA can be sticky to plastic tubes and we highly recommend using tubes that are low binding for nucleic acids. Also, please clearly label tubes so that they are legible and match the sample ID in the Project Information Form. Take care not to duplicate names within your project.

Quality control assays typically require 5 µL of sample. Please provide sufficient sample to account for QC and library construction. A preliminary ballpark estimate of concentration is helpful.

The GTC will perform quality control test(s) on all samples. For DNA-based libraries (e.g., whole genome sequencing and methyl-seq libraries), we will not run the gDNA on a fragment analyzer as these types of samples have previously been shown to diminish the life span/resolution of our fragment analyzer arrays (which we heavily rely upon for accuracy). These types of samples should be run on an agarose gel by the researcher submitting the samples to ensure the DNA is of good quality and free of significant amounts of RNA. The GTC does not have the bandwidth to run agarose gels and it must be done by the researcher. When the Project Information Form is submitted, please provide a picture of the agarose gel showing the condition of the DNA. Many DNA isolation protocols include the use of RNase to get rid of this component. Better quality DNA results in better quality DNA libraries. The integrity of total RNA will be checked on the Fragment Analyzer.

If there are any problems with your samples (poor quality RNA, low concentration RNA/DNA, etc.) we will contact you as soon as possible to confirm if you want to continue forward, replace/omit any samples, or put the project on hold.

Constructing your libraries.

If available supplies for constructing libraries are not on hand, they will be ordered when the samples are submitted to the GTC. This ensures fresh reagents are being used without wasting resources on unused kits. This is especially **important for single cell/nuclei and spatial transcriptomic projects** where reagents are even more expensive, have a relatively short shelf-life, and need to be ordered in time for the scheduled collection. For these reasons, it is important for us to know ahead of time your intentions for performing these experiments and why an ongoing discussion is essential. Shipments for these supplies are rarely delayed and why we try to schedule collections/processing at least 10 days in advance to account for any possible unforeseen interruptions. Attempts to schedule a single cell/nuclei collection without ample notification may result in loss of the sample you spent significant amounts of time and resources collecting.

Sequencing libraries.

A typical sequencing project takes approximately 4 weeks. You are welcome to email or come by to check on the progress of your project if you are curious. However, we ask that you take care when entering the lab to not interrupt someone actively working on a library prep. Processing a batch of samples requires focus on each sample and each step. We aim to devote our full attention to the

protocol and want to minimize any source of error. With that said, we would be happy to answer any questions when we are able to do so.

Getting access to the data and beyond.

Once the libraries have been sequenced and processed, a link will be sent to those listed on the Project Information Form to download the data. Our policy (https://mugenomicscore.missouri.edu/corepolicies.html) is to house the data for **6 months**. We do not have the resources to maintain data longer and it will not be available after this time. It is up to the researcher to make sure to save it to an appropriate location for long-term storage. If you need assistance with analysis of the data, please contact the Bioinformatics and Analytics Core.

Sanger sequencing.

A thorough description of submission requirements for Sanger sequencing can be found here: https://mugenomicscore.missouri.edu/sangersequencing.html

If you cannot find an answer to your question(s), please contact the GTC by sending an email to: mugenomicscore@missouri.edu.

A table to help keep organization of samples submitted:

Requisition number: _____ (record on bag when submitted)

Product/ plasmid	primer	Product concen. (ng/µL)	Mass to submit	Vol. of sample	Vol. of primer	Vol. of water	Final volume	Requisition number
Sample_plasmid	M13F	100	800	8	2	6	16 µL	123456
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	
							16 µL	

Whole genome sequencing (DNA libraries) and Methyl-seq.

A thorough description of submission requirements for DNA libraries and methyl-seq samples can be found here: https://mugenomicscore.missouri.edu/ngs.html

Project ID: _____

Resuspension buffer: _____

Date of agarose gel check: _____

Emailed agarose gel to GTC: ☐ yes ☐ oops!

Used RNase: ☐ yes ☐ oops!

Sample ID	Organism	Tissue	Extraction method	Concen.	volume

RNA-seq, small RNA, Chip-seq.

A thorough description of submission requirements for RNA-seq, small RNA and Chip-seq libraries can be found here: https://mugenomicscore.missouri.edu/ngs.html

Project ID: _____

Resuspension buffer: _____

For RNA samples: used DNase? ☐ yes ☐ no

Sample ID	Organism	Tissue	Extraction method	Concen.	volume

Single-cell/nuclei projects.

Submission requirements for single-cell/nuclei processing can be found here: https://mugenomicscore.missouri.edu/10xgenomics.html. Additionally, specific guidelines for processing 10x Genomics samples are supplied with the quote.

Project ID: _____

Scheduled collection date: _____ (establish 10-14 days in advance with GTC)

Number of washes: _____

Resuspension buffer: _____

Included RNase-inhibitor: ☐ yes ☐ no

Sample ID	Organism (*Genus species*)	Tissue/organ	Desired amount of cell/nuclei (max: 20k)

Spatial transcriptomics projects.

Submission requirements for spatial transcriptomics processing can be found here: https://mugenomicscore.missouri.edu/10xgenomics.html

Project ID: _____

Scheduled collection date: _____ (establish 10-14 days in advance with GTC)

Sectioning thickness: _____

Tissue capture area	Sample ID	Organism (*Genus species*)	Tissue/organ
A			
B			
C			
D			

Researcher prepared libraries.

Project ID: _____

Prep type: _____

Pool	Sample ID	Organism	Tissue/ organ	i7 Index	i5 Index	Prep date	Vol. (µL)	Conc.	Size (bp)

16S/ITS/Custom amplicon plates.
Project ID: _____
Profiling type: ☐ 16S ☐ ITS ☐ Custom
Fragment size: _____
Organism: _____
Plate ID: _____
Cycle number: _____ (default is 25)

Well	Sample	Well	Sample	Well	Sample	Well	Sample
A01		B01		C01		D01	
A02		B02		C02		D02	
A03		B03		C03		D03	
A04		B04		C04		D04	
A05		B05		C05		D05	
A06		B06		C06		D06	
A07		B07		C07		D07	
A08		B08		C08		D08	
A09		B09		C09		D09	
A10		B10		C10		D10	
A11		B11		C11		D11	
A12		B12		C12		D12	

Well	Sample	Well	Sample	Well	Sample	Well	Sample
E01		F01		G01		H01	
E02		F02		G02		H02	
E03		F03		G03		H03	
E04		F04		G04		H04	
E05		F05		G05		H05	
E06		F06		G06		H06	
E07		F07		G07		H07	
E08		F08		G08		H08	
E09		F09		G09		H09	
E10		F10		G10		H10	
E11		F11		G11		H11	
E12		F12		G12		H12	