

# FREQUENTLY ASKED QUESTIONS

## Table of Contents

Submission/Pricing.....	2
1. Can I get an estimate for my upcoming submission? .....	2
2. If some of my samples fail to amplify can the lab try other conditions to improve amplification? .....	2
3. Are there protocols I can follow to prepare my samples for submission? .....	2
4. Is there a form to fill out <b>prior</b> to submitting samples for sequencing? .....	2
5. How long will you keep my DNA (submitted or extracted)?.....	2
6. Can I use your services if I am not affiliated with the University of Michigan?.....	2
7. What is the MiSeq Nano Kit? .....	2
8. What is the minimum number of samples that I can send for sequencing? .....	3
9. How does the price differ if I have 1, 2, 3 plates for DNA extraction and 16S rDNA sequencing versus a full run (4 plates)?.....	3
10. How should I ship my plates?.....	3
11. Do you have a template for the plate map I need to submit electronically? .....	3
12. Why am I asked if I do metabolic mouse work on the submission form? .....	3
DNA Extraction.....	3
13. What extraction kit do you use? .....	3
14. Do I have to add my samples to the Bead Plate or do you do it? .....	4
15. How do I get a Bead Plate? .....	4
16. How much sample should I add to each well of the Bead Plate? .....	4
17. Where should I add my samples to the Bead Plate?.....	4
16S rDNA Sequencing .....	4
18. What region of 16S do you sequence and why?.....	4
19. What are your average reads per sample? .....	4
20. Do I have to pay for an entire plate if I am submitting less? .....	4
21. What if I want to test conditions prior to sending in my DNA? .....	4
22. Should I quantify my DNA? .....	4
23. What is touchdown PCR and how does it differ from standard PCR? .....	5
Genomic Libraries .....	5
24. Which kit do you use?.....	5
25. How much DNA do you need? .....	5
26. Do you sequence Eukaryotic genomes? .....	5
Data.....	6

27.	What is the Mock control and why do I need it? .....	6
28.	What type of data will I receive? .....	6
29.	Are the indices removed from the FASTQ files? .....	6
30.	How do I receive the data? .....	6
31.	Can I keep the USB? .....	6
32.	Why am I having trouble accessing my data on Basespace? .....	6
33.	What if I lose my data? .....	6
34.	What is in the workbook? .....	6
35.	How do most of your clients analyze their 16S rDNA data? .....	6
36.	Do you provide a service to analyze the data? .....	6
37.	How do I cite the HMI? .....	6

## Submission/Pricing

1. [Can I get an estimate for my upcoming submission?](#)  
Yes, please fill out this [estimate of costs form](#) and we will contact you shortly.
2. [If some of my samples fail to amplify can the lab try other conditions to improve amplification?](#)  
Yes, we will ask whether you would like us to troubleshoot your samples. Additional charges apply.
3. [Are there protocols I can follow to prepare my samples for submission?](#)  
Yes, please go to the bottom of our webpage to download all protocols.
4. [Is there a form to fill out prior to submitting samples for sequencing?](#)  
Yes. Sequencing submission form can be found [here](#).
5. [How long will you keep my DNA \(submitted or extracted\)?](#)  
We will keep DNA for 30 days after submission. After this time, samples are disposed of accordingly. We will ship your DNA back at cost to the customer or you can pick up DNA plates after the sequencing is done. We are not responsible for DNA that was not been claimed and was disposed of in the appropriate time. **It is best practice to take an aliquot of your DNA for submission and keep a set for yourself.**
6. [Can I use your services if I am not affiliated with the University of Michigan?](#)  
Yes, we do take external clients.
7. [What is the MiSeq Nano Kit?](#)  
The MiSeq Nano kit is a smaller version of the full 500-cycle Illumina kit we use for 16S rRNA gene sequencing. It will yield fewer reads than ¼ run of the full kit, but enough for data analysis. This kit offers a new route for external clients, as previously they were required to pay for a full run regardless of the number of plates they submitted. It also offers options for internal clients submitting a single plate and do not wish to wait for additional plates or have an upcoming deadline.

8. What is the minimum number of samples that I can send for sequencing?

We charge and process projects per plate (regardless if you have less than 96 samples).

	<b>University of Michigan Client</b>	<b>External Client</b>
<b>DNA extraction</b>	Cost is per plate*	Cost is per plate*
<b>16S rDNA sequencing</b>	Cost is per plate**	Cost is per plate**
<b>Background Genome</b>	Less than 6 genomes	Less than 6 genomes – only in your own low-diversity run (such as 16S)
<b>Full Genomic Run</b>	Cost is per plate	Cost is per plate

\*A plate is up to 93 samples + 3 library/extraction controls

\*\*A plate is up to 94 samples + 2 library/extraction controls

9. How does the price differ if I have 1, 2, 3 plates for DNA extraction and 16S rDNA sequencing versus a full run (4 plates)?

	<b>Full run (4 plates)</b>	<b>Partial Runs</b>	<b>One plate</b>
<b>DNA extraction</b>	Charge per plate, 4 plates	Charge per plate- 1, 2 or 3 plates	1 plate
<b>16S rDNA library prep</b>	Charge per plate, 4 plates	Charge per plate- 1, 2 or 3 plates	1 plate
<b>Sequencing</b>	100%	100%	100% MiSeq Nano 500-V2 Sequencing kit

10. How should I ship my plates?

Please use and follow our submission protocols. You can find all of them at the bottom of our webpage.

11. Do you have a template for the plate map I need to submit electronically?

Yes, the plate map can be found [here](#). NOTE: Each cell MUST have a unique name containing no special characters; use only numbers and letters. SPECIAL CHARACTERS INCLUDE: + ! , @ # \$ % ^ & \* ( ) . < > ? / { } [ ] \ | : ' \* - " ~ =

12. Why am I asked if I do metabolic mouse work on the submission form?

We are part of the National Mouse Metabolic Phenotyping Centers (MMPC). The MMPC is a NIH-sponsored resource that provides experimental testing services to scientists studying diabetes, obesity, diabetic complications, and other metabolic diseases in mice. By initiating your order through the MMPC, your submission ensures continued financial support for the Core services our lab provides.

## DNA Extraction

13. What extraction kit do you use?

Qiagen MagAttract PowerMicrobiome Kit, this was previously called MoBio PowerMag Microbiome kit. For more details, the manual can be found [here](#).

14. Do I have to add my samples to the Bead Plate or do you do it?

Yes, we do not add samples to the Bead Plate.

15. How do I get a Bead Plate?

If you are on campus, please come by the lab located in MSRB1 Room 1500 (call 734-615-2958 to ensure someone is in the lab for pick up). If you are external, please let us know how many Bead Plates you would like shipped to you. We will package them and send you the info with the package dimensions and weight. You can then send us a PDF shipping label through the shipping provider of your choice. Once filled you can send the Bead Plates back on dry ice.

16. How much sample should I add to each well of the Bead Plate?

250 uL or 0.25 g can be added to each well. Please seal well with the silicone mat provided before sending back and wrap them with parafilm.

17. Where should I add my samples to the Bead Plate?

A biohazard hood using appropriate personal protective equipment.

## 16S rDNA Sequencing

18. What region of 16S do you sequence and why?

We amplify the V4 region of the 16S rRNA gene using barcoded dual-index primers developed in the lab of [Pat Schloss](#). In the posts listed below, he describes why this method was selected and why larger regions or variants of this region are not used. Briefly, if the reads do not fully overlap, your assembled reads will have a high error rate.

<http://blog.mothur.org/2016/07/07/Customization-for-your-region/>

<http://blog.mothur.org/2014/09/11/Why-such-a-large-distance-matrix/>

19. What are your average reads per sample?

For samples that have amplified well we average over 15000 reads/sample. However, this cannot be guaranteed as many factors influence average reads/sample. For additional information about sequencing coverage: [https://support.illumina.com/downloads/sequencing\\_coverage\\_calculator.html](https://support.illumina.com/downloads/sequencing_coverage_calculator.html)

20. Do I have to pay for an entire plate if I am submitting less?

Yes, if you do not have an entire plate you are still responsible for the cost of the entire plate, but you will receive more coverage of those samples than if you submitted more samples.

21. What if I want to test conditions prior to sending in my DNA?

Please let us know if you would like primers to test amplification. In addition to the primers you may want to use the same polymerase, AccuPrime Taq DNA High Fidelity Polymerase (Thermo Fisher Scientific, catalog no. 12346094). Please see our protocols for a full outline of methods used.

22. Should I quantify my DNA?

If we are extracting your samples, we will quantify your samples and this is included in our services. If you are sending extracted DNA you should quantify your samples and send them with concentrations between 1-5ng/uL. Please use a fluorescence-based assay such as Picogreen or Qubit **not Nanodrop**. We can also quantify your gDNA for an additional charge.

23. What is touchdown PCR and how does it differ from standard PCR?

**Standard:**

Temp	Time	Cycles
95	2 m	1 x
95	20 s	30 x
55	15 s	
72	5 m	
72	10 m	1 x
4	forever	

**Touchdown:**

Temp	Time	Cycles
95	2 m	1 x
95	20s	20 x (temp increase -0.3)
60	15s	
72	5m	
95	20 s	20 x
55	15 s	
72	5 m	
72	10 m	1 x
4	forever	

The touchdown program is modified from the standard program as the initial annealing temperature is higher than the optimal  $T_m$  of the primers and is gradually reduced over subsequent cycles until the  $T_m$  temperature or “touchdown temperature” is reached. This increases specificity and sensitivity in PCR amplification (See this [paper](#)). Additionally, after 20 rounds of touchdown PCR, another 20 rounds of standard PCR are performed. Touchdown is a great tool for low biomass samples, but should be used with caution as any trace amounts of 16S will amplify including that in the negative controls.

## Genomic Libraries

24. Which kit do you use?

We use the Illumina NexteraXT kit (until Nov 2018) and the Illumina Nextera Flex kit. If you are sequencing numerous genomes and require a full sequence run, you may choose which sequencing kit you would like to use (2x250bp V2, 2x300bp V3). You could also sequence the final library in another sequencing facility.

25. How much DNA do you need?

We prefer to get 20uL of sample with concentrations between 1-5 ng/uL.

26. Do you sequence Eukaryotic genomes?

No, only microbial genomes.

## Data

### 27. What is the Mock control and why do I need it?

The mock community used is commercially produced ZymoBIOMICS Microbial Community DNA Standard (Zymo Research cat# D6306), which is a mixture of genomic DNA extracted from pure cultures of eight bacterial and two fungal strains. This mock community can be used for error analysis (see: [Mothur Wiki](#)). The FASTA file for this mock can be found [here](#).

### 28. What type of data will I receive?

FASTQ files

### 29. Are the indices removed from the FASTQ files?

Yes.

### 30. How do I receive the data?

We can either transfer the run/project to you over [basespace.illumina.com](https://basespace.illumina.com) or provide you with a USB containing your data.

### 31. Can I keep the USB?

Yes, it is include in the cost of your run.

### 32. Why am I having trouble accessing my data on Basespace?

You need to open an account using your email and provide us that email to transfer your data.

### 33. What if I lose my data?

We do keep backups of data, so if you lose your USB or delete your basespace run we will likely have a backup copy. A fee of \$75 will be charged to retrieve your data.

### 34. What is in the workbook?

The workbook contains your plate map, PCR conditions for each of your samples, any troubleshooting conditions, summary of run metrics, how your samples indexed and a methods sheet.

### 35. How do most of your clients analyze their 16S rDNA data?

Most of our clients use Mothur to analyze their data. The [MiSeq SOP](#) on the Mothur wiki page is an excellent resource. Additionally, Pat Schloss offers numerous [workshops](#) throughout the year.

### 36. Do you provide a service to analyze the data?

We do not currently provide analytical support for external clients at this time. For clients within the University of Michigan Medical School, we can provide analytical support.

### 37. How do I cite the HMI?

We ask that all publications and presentations of research results supported by the Microbial Systems Molecular Biology Laboratory (MSMBL) contain the following (or equivalent) acknowledgement: "This research was supported by work performed by The University of Michigan Microbial Systems Molecular Biology Laboratory"

Please let us know as soon as you have an article accepted for publication, poster, or meeting presentation which has received MSMBL support. Early notification helps us to track the activities being supported by the MSMBL. Submit your notifications by email to [msmbcore@umich.edu](mailto:msmbcore@umich.edu). Be sure to include a copy of the presentation abstract or complete accepted article.