Submission of DNA for 16S-V4, 16S-V3V4 amplicon preparation

Sample submission requirements for 16S amplicon libraries and other amplicon libraries. The 16S-V4 or 16S-V3V4 library preparation is a one-step PCR library preparation, all other amplicons are 2-step PCR library preps, therefore the requirements are different.

For 16S-V4 or 16S-V3V4, submitters must:
-quantify DNA via Qubit (or other fluorometric method) and normalize all samples to the same concentration.
-Perform a test amplification on all samples to demonstrate: the target region is able to amplify and the product is the expected size. The gel must be included with the submission. If a dilution of the samples was required, please provide that information as well.
-provide samples at a minimum concentration of 1ng/ul in a minimum volume of 10ul.
-submit in 96-well plate(s), partially filled plates must be filled by column, not row.

For the amplification testing of 16S V4, any validated pair of 16S PCR primers may be used. If you wish to use the same primers which we will use for 16S-V4 amplicons, 515f/806r (Kozich et al. 2013) they are:

16S V4 forward (515f): 5'-GTGCCAGCMGCCGCGGTAA
16S V4 reverse (806r): 5'-GGACTACHVGGGTWTCTAAT

Library prep for 16S-V4 or 16S-V3V4 libraries is $8 each. The libraries are then normalized using Invitrogen DNA normalization plates and recovered products are pooled. The pool is QC'd, there is a $20 charge for this.

Available barcodes (i.e. maximum number of samples combined in one run)
16S-V4 576 using dual-indexed adapters
16S-V3V4 96 using single-indexed adapters

Preparing other amplicons for submission; index adapters added by MSU Genomics Core.

For all other amplicon sequencing, the first PCR uses target-specific primers with tags on the 5' ends that allow us to do a second PCR for barcoding. Submitters must perform the primary PCR reaction. To do this you will need to order primers with the following tags on the 5' ends, where you will insert your desired target specific forward and reverse primers in [TS-For] and [TS-Rev], respectively:

CS1-TS-F: 5'- ACACTGACGACATGGTTCTACA – [TS-For] – 3'
CS2-TS-R: 5'- TACGGTAGCAGAGACTTGGTCT – [TS-Rev] – 3'

Our submission requirements are as follows:
-quantify via Qubit (or other fluorometric method) and normalize all samples to the same concentration.
-Provide a gel image of the products to demonstrate that target region was successfully amplified and the product is the expected size. The gel must be included with the submission.
-minimum concentration of 5ng/ul
-minimum volume of 10ul
-submit in 96-well plate(s), partially filled plates must be filled by column, not row

The cost to have primary PCR products barcoded is $8 per sample. After barcoding, the libraries are normalized using Invitrogen DNA normalization plates and recovered products are pooled. The pool is QC'd, there is a $20 charge for this.

Available barcodes (i.e. maximum number of samples combined in one run)
All custom amplicons 576 using dual-indexed adapters

One MiSeq v2 standard 500cycle 2x250bp run will cost $1,376. There is a 2% administrative fee for forms of payment other than MSU accounts. The MiSeq v2 standard 500cycle 2x250bp run will output approximately 8-10M read pairs when amplicon sequencing is performed.