**Fluidigm Access Array Ribosomal Amplicon Assays**

**Requirements for submission**

**Extraction protocols** – We recommend MO BIO bacterial or soil DNA extraction kits. Other protocols are also acceptable.

**DNA Quality**- We request submission of a gel picture showing high molecular weight Genomic DNA.

**Amount to submit**- Ideally, at least 15 ul of 20-40 ng/ul if measuring with nanodrop. (Please note that nanodrop measurements below 20 ng/ul are extremely inaccurate). We will use 2 ul for Qubit high sensitivity concentration measures and dilute to working concentration.

260/230 and 260/280 ratios should optimally be between 1.7-2.0. Ratios diverging from this range may contain contaminants or proteins that can affect the efficiency of PCR amplification.

Samples must be supplied in well-sealed 96 well PCR plates with 48 samples in the **first 6 columns. Please use any cone shaped well, no flat bottom wells.**

**An excel spreadsheet with the plate layout and sample IDs is required.**

**A unique ID is required for each well. Please label replicate samples with A or B extentions.**

**Shipping**- Samples should be shipped overnight on dry ice to the following address:

Functional Genomics Laboratory

University of Illinois

1201 W. Gregory Dr.

ERML 356

Urbana, IL 61801

Tel: 217 244 3930 Attn: Mark Band

Please provide shipper and tracking number to [markband@illinois.edu](mailto:markband@illinois.edu) or [akraiko@illinois.edu](mailto:akraiko@illinois.edu) .

Please package plates in appropriate packing material to avoid damage or leakage.

**Number of samples**

Access Arrays are run with up to 48 samples each. No discounts will be credited for fewer than 48 samples.

**Primer choices**- Target Specific Primer 16s or 18S regions are listed below. (We also have a number of targets for soil samples that may be of interest. Custom primers can be designed and the researcher will be charged an additional $100 for synthesis, shipping, and testing for the first 3 primer sets and $50 for each additional set, prior to Access Array run. We currently can run Fluidigm Access Arrays at annealing temperature of 60C or 55C so Tm of custom primers should be targeted appropriately. Note that all primers are run simultaneously with the same PCR cycling program.

**Default Primer Options (all primers are run by default at annealing 550 C)**

**Please note that the bioanalyser sizes are about 104 bases longer than the actual sequenced amplicon, due to the Fluidigm and Illumina linker extensions.**

**Fluidigm CS1 Adapter**

**Fluidigm CS2 Adapter**

**Underlined = Target Primer Region**

**Please note the revision to the V4 primers. By default we will be using these revised primers unless client specifically requests the old set.**

**V1-V3 643 bp Bioanalyzer**

**CS1-V1-V3 F28-2-for ACACTGACGACATGGTTCTACAGAGTTTGATCNTGGCTCAG**

**CS2-V1-V3 R519-2-rev TACGGTAGCAGAGACTTGGTCTGTNTTACNGCGGCKGCTG**

**V3-V5 694 bp Bioanalyzer**

**CS1-V3-V5 F357-1-for ACACTGACGACATGGTTCTACACCTACGGGAGGCAGCAG**

**CS2-V3-V5 R926-1-rev TACGGTAGCAGAGACTTGGTCTCCGTCAATTCMTTTRAGT**

**V3-V4 ~540 bp Bioanalyzer**

**V3-357F ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCAG**

**V4-805R TACGGTAGCAGAGACTTGGTCTGACTACHVGGGTATCTAATCC**

**V4 ~400 bp Bioanalyzer (Old set)**

**V4-515F ACACTGACGACATGGTTCTACAGTGCCAGCMGCCGCGGTAA**

**V4-806R TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCTAAT**

**V4 (revised) ~400 bp Bioanalyzer (The earth microbiome consortium has recently recommended these changes**

**V4-515F ACACTGACGACATGGTTCTACAGTGYCAGCMGCCGCGGTAA**

**V4-806R TACGGTAGCAGAGACTTGGTCTGGACTACNVGGGTWTCTAAT**

**Archaea 528 bp Bioanalyzer**

**CS1-Arch349F-3-for ACACTGACGACATGGTTCTACAGYGCASCAGKCGMGAAW**

**CS2-Arch806R-3-rev TACGGTAGCAGAGACTTGGTCTGGACTACVSGGGTATCTAAT**

**Euk566 765 + bp Bioanalyzer**

**CS1-F566Euk-4-for ACACTGACGACATGGTTCTACACAGCAGCCGCGGTAATTCC**

**CS2-R1200Euk-4-rev TACGGTAGCAGAGACTTGGTCTCCCGTGTTGAGTCAAATTAAGC**

**ITS1-ITS4 580 + bp Bioanalyzer**

**CS1-ITS1-10-for ACACTGACGACATGGTTCTACATCCGTAGGTGAACCTGCGG**

**CS2-ITS4-10-rev TACGGTAGCAGAGACTTGGTCTTCCTCCGCTTATTGATATGC**

**ITS3-ITS4 462 + bp Bioanalyzer**

**CS2-ITS3F-8-for ACACTGACGACATGGTTCTACAGCATCGATGAAGAACGCAGC**

**CS2-ITS4R-10-rev TACGGTAGCAGAGACTTGGTCTTCCTCCGCTTATTGATATGC**

**18S 200-279 bp Bioanalyzer**

**CS1-Euk\_1391f-7-for ACACTGACGACATGGTTCTACAGTACACACCGCCCGTC**

**CS2-EukBr-7-rev TACGGTAGCAGAGACTTGGTCTTGATCCTTCTGCAGGTTCACCTAC**

Below are Bioanalyzer profiles for a number of primer pairs listed above. Fragment sizes are approximately 105 bp longer than the targeted amplicon due to the Fluidigm CS1 and CS2 tails as well as the Illumina barcodes and linkers. Note that certain metacommunities may show varying amplicon lengths.













