Amplicon (16S rRNA):

For V3 and V4 16S rRNA chemistry: DNA is extracted using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen; 47016). Libraries are made using a modification of the Illumina 16S Metagenomic Sequencing Library Preparation workflow. Briefly, 12.5 ng of DNA is amplified using 16S Amplicon PCR Forward and Reverse Primers; libraries are purified with Ampure XP beads (Beckman; A63880); purified amplicons are indexed with Nextera XT Index primers (Illumina; FC-131-1001 or FC-131-1002); indexed amplicons are purified with Ampure XP beads. Final 16S libraries are approximately 630 bp and are pooled in equal amounts based on fluorescence quantification. Final library pools are quantitated via qPCR (Kapa Biosystems; KK4824). The pooled library is sequenced on an Illumina MiSeq using MiSeq v3 600 cycle chemistry (Illumina; MS-102-3003) at a loading density of 6-8 pM with 20% PhiX, generating roughly 20 million, 300 bp paired-end reads. Recommended sequencing depth is >100,000 reads per sample.

16S Amplicon PCR Forward Primer
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3'
16S Amplicon PCR Reverse Primer
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'

For V4 16S rRNA chemistry using GoLay barcodes: DNA is isolated using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen; 47016). The V4 16S rRNA gene region is amplified using 12.5 ng of DNA with primers 515F and 806R as previously described (Caporaso et al 2012). Resulting amplicons are ~350 bp. 16S Amplicons are purified with Ampure XP beads (Beckman; A63880) and pooled in equal amounts based on fluorescent quantification. Final library pools are quantitated via qPCR (Kapa Biosystems; KK4824). Sequencing is performed on an Illumina MiSeq using MiSeq v2 500 cycle chemistry (Illumina; MS-102-2003) at a loading density of 8 pM with 20% PhiX, generating roughly 16 million 250 bp paired-end reads.

515f PCR Primer Sequence – Forward primer
5'-AATGATACGGCGACCACCGAGATCTACAC TATGGTAATT GT GTGCCAGCMGCCGCGGTAA-3'
806r PCR primer sequence – Reverse primer, barcoded
5'-CAAGCAGAAGACGGCATACGAGAT XXXXXXXXXXXX AGTCAGTCAG CC GGACTACHVGGGTWTCTAAT-3'