

Table 3 Primers and cycling protocol for 16S rRNA targeting V3 region with specific adapters

Primers	Sequence	Size (bp)	Cycling protocol	References
Forward V3_F	aatgatacggcgaccaccgagatctacacttttccctacacgagctc <u>ttccgctct</u> CCTACGGGAGGCAGCAG			
Reverse V3_2R	caagcagaagacggcatacagagat ACATCG <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_3R	caagcagaagacggcatacagagat GCCTAA <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_4R	caagcagaagacggcatacagagat TGGTCA <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_6R	caagcagaagacggcatacagagat ATTGGC <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_7R	caagcagaagacggcatacagagat GATCTG <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_8R	caagcagaagacggcatacagagat TCAAGT <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_9R	caagcagaagacggcatacagagat CTGATC <u>gtgactggagttca</u> <u>gacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_10R	caagcagaagacggcatacagagat AAGCTA <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG	330	Cycling of 95 °C for 5 min, 20 cycles of 98 °C for 20 sec, 58 °C for 15 sec and 72 °C for 5 sec, then 72 °C for 5min and hold at 10 °C	Bartram <i>et al.</i> , 2011
V3_11R	caagcagaagacggcatacagagat GTAGCC <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_12R	caagcagaagacggcatacagagat TACAAG <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_13R	caagcagaagacggcatacagagat CGTACT <u>gtgactggagttca</u> <u>gacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_14R	caagcagaagacggcatacagagat GACTGA <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_15R	caagcagaagacggcatacagagat GCTCAA <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_16R	caagcagaagacggcatacagagat TCGCTT <u>gtgactggagttca</u> <u>gacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_22R	caagcagaagacggcatacagagat CTCTCT <u>gtgactggagttca</u> <u>gacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			
V3_24R	caagcagaagacggcatacagagat CCTTGA <u>gtgactggagttc</u> <u>agacgtgtgctcttccgatct</u> ATTACCGCGGCTGCTGG			

Note: Lowercase letter denote the adapter sequences necessary for binding to the flow cell, underlined lowercase are binding sites for the Illumina sequencing primers, bold uppercase highlights the index sequences and regular uppercase are the V3 region primers (341F on for the forward primers and 518R for the reverse primers)