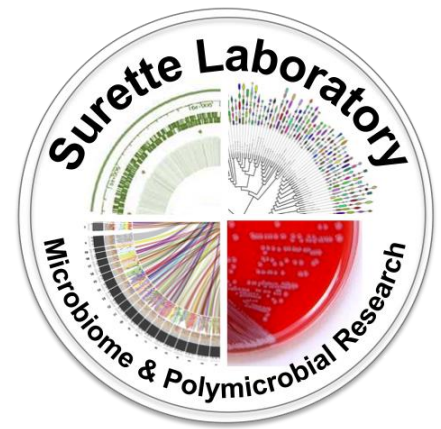

PCR AMPLIFICATION OF THE 16S rRNA V34 REGION FOR ILLUMINA SEQUENCING



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BACKGROUND

- This protocol is heavily adapted from that described in Bartam *et al.*, and is used to PCR amplify the variable regions 3 and 4 of the 16S rRNA gene whilst including up to 96 unique barcode tags, 1 per sample, in order to multiplex samples in a manner compatible with the Illumina technologies.

EQUIPMENT

- Thermal cycler (Veriti 96 well thermal cycler, Applied Biosystems, #437586)
- PCR Workstation (CanadaWide Scientific, #95-0438-01)

PROTOCOL

- In a sterile, PCR workstation combine the following per sample:

Reaction Mix

- 5 μ l 10x PCR buffer (Life Technologies, #10342020)
 - 2 μ l 10mg/mL bovine serum albumin (made up with water and irradiated with UV for 15min to get rid of contaminating DNA.)
 - 1.5 μ l 50mM MgCl₂ (Life Technologies, #10342020)
 - 1 μ l 10mM dNTPs (New England Biolabs, #N0447L)
 - 5 μ l 1 μ M V3F_mod2ver2 barcoded primer (5 pmoles) (*see Appendix A for barcodes and adapters, see below for 16S portion*)
 - 5 μ l 1 μ M V4Rver2 primer (5 pmoles) (*see Appendix A for barcodes and adapters, see below for 16S portion*)
 - x μ l dH₂O, up to 50 μ l
 - 0.25 μ l Taq polymerase (Life Technologies, #10342020) *If having trouble with getting a product, can increase to 0.5 μ l*
 - x μ l template DNA (30ng)
- 50 μ l total
- Transfer 2x 16.7 μ l to 2 new tubes for a reaction in triplicate.

PRIMERS

v3f_341f – CCTACGGGNGGCWGCAG – as per Illumina protocol

v4r_806r – GGACTACNVGGGTWTCTAAT – as per Earth Microbiome protocol

- PCR amplify using the following program:

Program

94°C – 5min

CYCLE (5 CYCLES):

94°C – 30s

47°C – 30s

72°C – 40s

CYCLE (25 CYCLES):

94°C – 30s

50°C – 30s

72°C – 40s

END CYCLE

72°C – 10min

4°C - Infinity

Run 5uL of the reaction on a 1.5-2% gel to visualize the expected 600 base pair product.

REFERENCES

- Bartram *et al.* **Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads.** Appl Environ Microbiol. 2011 Jun;77(11):3346-52.

APPENDIX A – FOR ADAPTERS AND BARCODES

PRIMERS: 16S rRNA V3 REGION REVERSE PRIMER

Primer	Adaptor	Sequence
V3_Rmod2	P5	aatgatacggcgaccaccgagatctacactctttccctacacgacgctcttccgatctNNNNATTACCGG GCTGCTGG

PRIMERS: 16S rRNA V3 REGION FORWARD BARCODED PRIMER

Primer	Adaptor	Sequence
V3_1F	P7	caagcagaagacggcatacagagat CGTGAT gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_2F	P7	caagcagaagacggcatacagagat ACATCG gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_3F	P7	caagcagaagacggcatacagagat GCCTAA gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_4F	P7	caagcagaagacggcatacagagat TGGTCA gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_5F	P7	caagcagaagacggcatacagagat CACTGT gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_6F	P7	caagcagaagacggcatacagagat ATTGGC gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_7F	P7	caagcagaagacggcatacagagat GATCTG gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_8F	P7	caagcagaagacggcatacagagat TCAAGT gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_9F	P7	caagcagaagacggcatacagagat CTGATC gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_10F	P7	caagcagaagacggcatacagagat AAGCTA gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_11F	P7	caagcagaagacggcatacagagat GTAGCC gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG

V3_12F	P7	caagcagaagacggcatacagagat TACAAG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACG GGAGGCAGCAG
V3_13F	P7	caagcagaagacggcatacagagat CGTACT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_14F	P7	caagcagaagacggcatacagagat GACTGA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACG GGAGGCAGCAG
V3_15F	P7	caagcagaagacggcatacagagat GCTCAA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_16F	P7	caagcagaagacggcatacagagat TCGCTT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_17F	P7	caagcagaagacggcatacagagat TGAGGA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACG GGAGGCAGCAG
V3_18F	P7	caagcagaagacggcatacagagat ACAACC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_19F	P7	caagcagaagacggcatacagagat ACCTCA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_20F	P7	caagcagaagacggcatacagagat ACGGTA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACG GGAGGCAGCAG
V3_21F	P7	caagcagaagacggcatacagagat AGTTGG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACG GGAGGCAGCAG
V3_22F	P7	caagcagaagacggcatacagagat CTCTCT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_23F	P7	caagcagaagacggcatacagagat CAAGTG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACG GGAGGCAGCAG
V3_24F	P7	caagcagaagacggcatacagagat CCTTGA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_25F	P7	caagcagaagacggcatacagagat ACCACT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_26F	P7	caagcagaagacggcatacagagat AGTGTC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_27F	P7	caagcagaagacggcatacagagat AGAAGG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACG

		GGAGGCAGCAG
V3_28F	P7	caagcagaagacggcatacagagat TTATCC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_29F	P7	caagcagaagacggcatacagagat TAAGG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_30F	P7	caagcagaagacggcatacagagat TTCTTG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_31F	P7	caagcagaagacggcatacagagat TCAAC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_32F	P7	caagcagaagacggcatacagagat TTGTGA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_33F	P7	caagcagaagacggcatacagagat TTGACT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_34F	P7	caagcagaagacggcatacagagat TATTCG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_35F	P7	caagcagaagacggcatacagagat TATAGC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_36F	P7	caagcagaagacggcatacagagat TAACTC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_37F	P7	caagcagaagacggcatacagagat TACCAA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_38F	P7	caagcagaagacggcatacagagat TACGTT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_39F	P7	caagcagaagacggcatacagagat TAGTAC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_40F	P7	caagcagaagacggcatacagagat TAGATG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_41F	P7	caagcagaagacggcatacagagat TCTACA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_42F	P7	caagcagaagacggcatacagagat TCTGAT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG

V3_43F	P7	caagcagaagacggcatacagagat TCATGT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_44F	P7	caagcagaagacggcatacagagat TGTCTA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_45F	P7	caagcagaagacggcatacagagat ATTCTC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_46F	P7	caagcagaagacggcatacagagat ATTGAG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_47F	P7	caagcagaagacggcatacagagat ATACCT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_48F	P7	caagcagaagacggcatacagagat ATGCAA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_49F	P7	caagcagaagacggcatacagagat AATCCA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_50F	P7	caagcagaagacggcatacagagat AATGGT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_51F	P7	caagcagaagacggcatacagagat AACTAG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_52F	P7	caagcagaagacggcatacagagat AACACT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_53F	P7	caagcagaagacggcatacagagat AAGAGA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_54F	P7	caagcagaagacggcatacagagat ACTTAC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_55F	P7	caagcagaagacggcatacagagat ACATTG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_56F	P7	caagcagaagacggcatacagagat ACGAAT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_57F	P7	caagcagaagacggcatacagagat AGTCAT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_58F	P7	caagcagaagacggcatacagagat AGAAGT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG

		GGAGGCAGCAG
V3_59F	P7	caagcagaagacggcatacagagat CTTATG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_60F	P7	caagcagaagacggcatacagagat CTAGAA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_61F	P7	caagcagaagacggcatacagagat CATCTT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_62F	P7	caagcagaagacggcatacagagat CACATA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_63F	P7	caagcagaagacggcatacagagat CCAATT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_64F	P7	caagcagaagacggcatacagagat CGATTA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_65F	P7	caagcagaagacggcatacagagat GTTAGT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_66F	P7	caagcagaagacggcatacagagat GTAAC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_67F	P7	caagcagaagacggcatacagagat GTGTAT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_68F	P7	caagcagaagacggcatacagagat GATAAG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_69F	P7	caagcagaagacggcatacagagat GAATCT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_70F	P7	caagcagaagacggcatacagagat TTCCGT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_71F	P7	caagcagaagacggcatacagagat TTCGCA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_72F	P7	caagcagaagacggcatacagagat TTGGTC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_73F	P7	caagcagaagacggcatacagagat TGACAG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG

V3_74F	P7	caagcagaagacggcatacagagat ATCTGC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_75F	P7	caagcagaagacggcatacagagat ACACGA <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_76F	P7	caagcagaagacggcatacagagat AGGTTC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_77F	P7	caagcagaagacggcatacagagat CATGAC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_78F	P7	caagcagaagacggcatacagagat GCTATC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_79F	P7	caagcagaagacggcatacagagat GGACTT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_80F	P7	caagcagaagacggcatacagagat GGCAAT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_81F	P7	caagcagaagacggcatacagagat TCTCGG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_82F	P7	caagcagaagacggcatacagagat TCAGCG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_83F	P7	caagcagaagacggcatacagagat TGTGCC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_84F	P7	caagcagaagacggcatacagagat TGCACG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_85F	P7	caagcagaagacggcatacagagat AAGGCC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_86F	P7	caagcagaagacggcatacagagat ACCAGG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_87F	P7	caagcagaagacggcatacagagat AGCCTG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_88F	P7	caagcagaagacggcatacagagat AGCGAC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_89F	P7	caagcagaagacggcatacagagat CTACGC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG

		GAGGCAGCAG
V3_90F	P7	caagcagaagacggcatacagagat CTCCAG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GAGGCAGCAG
V3_91F	P7	caagcagaagacggcatacagagat CCGTAG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_92F	P7	caagcagaagacggcatacagagat CGGTGT <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_93F	P7	caagcagaagacggcatacagagat CGGAAC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_94F	P7	caagcagaagacggcatacagagat GTGCTG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_95F	P7	caagcagaagacggcatacagagat GAACGG <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG
V3_96F	P7	caagcagaagacggcatacagagat GGATGC <u>gtgactggagttcagacgtgtgctcttccgatct</u> CCTACGG GGAGGCAGCAG