ILLUMINA PCR AMPLIFICATION OF THE 16S RRNA V3 REGION

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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 3 region 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

- 5µl 10x PCR buffer (Life Technologies, #10342020)
- 2µl 10mg/mL bovine serum albumin (made up with water and irradiated with UV for 15min to get rid of contaminating DNA.)
- 1.5µl 50mM $MgCl_2$ (Life Technologies, #10342020)
- 1µl 10mM dNTPs (New England Biolabs, #N0447L)
- 5µl 1µM V3F_mod2 barcoded primer (5 pmoles) (*see Appendix A*)
- 5µl 1µM V3R primer (5 pmoles) (*see Appendix A*)
- xµl dH2O, up to 50ul
- 0.25µl Taq polymerase (Life Technologies, #10342020) *If having trouble with getting a product, can increase to 0.5µl*
- xµl template DNA (30ng)

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50µl total

- Transfer 2x 16.7µl to 2 new tubes for a reaction in triplicate.

- PCR amplify using the following program:

Program

94°C – 2min
CYCLE (30 CYCLES):
94°C – 30s
50°C – 30s
72°C – 30s
END CYCLE
72°C – 10min
4°C - Infinity

Run 5uL of the reaction on a 1.5-2% gel to visualize the expected 300 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

PRIMERS: 16S RRNA V3 REGION REVERSE PRIMER

Primer	Adaptor	Sequence
V3_Rmod2	P5	aatgatacggcgaccaccgagatctacactctttccctacacgacgctcttccgatctNNNNATTACCGCGGCTGCTGG

PRIMERS: 16S RRNA V3 REGION FORWARD BARCODED PRIMER

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

V3_12F	P7	caagcagaagacggcatacgagat**TACAAG**gtgactggagttcagacgtgtgctcttccgatctCCTACG GGAGGCAGCAG
V3_13F	P7	caagcagaagacggcatacgagat**CGTACT**gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_14F	P7	caagcagaagacggcatacgagat**GACTGA**gtgactggagttcagacgtgtgctcttccgatctCCTACG GGAGGCAGCAG
V3_15F	P7	caagcagaagacggcatacgagat**GCTCAA**gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_16F	P7	caagcagaagacggcatacgagat**TCGCTT**gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_17F	P7	caagcagaagacggcatacgagat**TGAGGA**gtgactggagttcagacgtgtgctcttccgatctCCTACG GGAGGCAGCAG
V3_18F	P7	caagcagaagacggcatacgagat**ACAACC**gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_19F	P7	caagcagaagacggcatacgagat**ACCTCA**gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_20F	P7	caagcagaagacggcatacgagat**ACGGTA**gtgactggagttcagacgtgtgctcttccgatctCCTACG GGAGGCAGCAG
V3_21F	P7	caagcagaagacggcatacgagat**AGTTGG**gtgactggagttcagacgtgtgctcttccgatctCCTACG GGAGGCAGCAG
V3_22F	P7	caagcagaagacggcatacgagat**CTCTCT**gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_23F	P7	caagcagaagacggcatacgagat**CAAGTG**gtgactggagttcagacgtgtgctcttccgatctCCTACG GGAGGCAGCAG
V3_24F	P7	caagcagaagacggcatacgagat**CCTTGA**gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_25F	P7	caagcagaagacggcatacgagat**ACCACT**gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_26F	P7	caagcagaagacggcatacgagat**AGTGTC**gtgactggagttcagacgtgtgctcttccgatctCCTACGG GAGGCAGCAG
V3_27F	P7	caagcagaagacggcatacgagat**AGAAGG**gtgactggagttcagacgtgtgctcttccgatctCCTACG

		GGAGGCAGCAG
V3_28F	P7	caagcagaagacggcatacgagat**TTATCC**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_29F	P7	caagcagaagacggcatacgagat**TTAAGG**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_30F	P7	caagcagaagacggcatacgagat**TTCTTG**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_31F	P7	caagcagaagacggcatacgagat**TTCAAC**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_32F	P7	caagcagaagacggcatacgagat**TTGTGA**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_33F	P7	caagcagaagacggcatacgagat**TTGACT**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_34F	P7	caagcagaagacggcatacgagat**TATTCG**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_35F	P7	caagcagaagacggcatacgagat**TATAGC**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_36F	P7	caagcagaagacggcatacgagat**TAACTC**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
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V3_40F	P7	caagcagaagacggcatacgagat**TAGATG**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
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V3_70F	P7	caagcagaagacggcatacgagat**TTCCGT**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_71F	P7	caagcagaagacggcatacgagat**TTCGCA**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
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V3_74F	P7	caagcagaagacggcatacgagat**ATCTGC**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
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		GAGGCAGCAG
V3_90F	P7	caagcagaagacggcatacgagat**CTCCAG**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
V3_91F	P7	caagcagaagacggcatacgagat**CCGTAG**gtgactggagttcagacgtgtgctcttccgatctCCTACGGGAGGCAGCAG
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