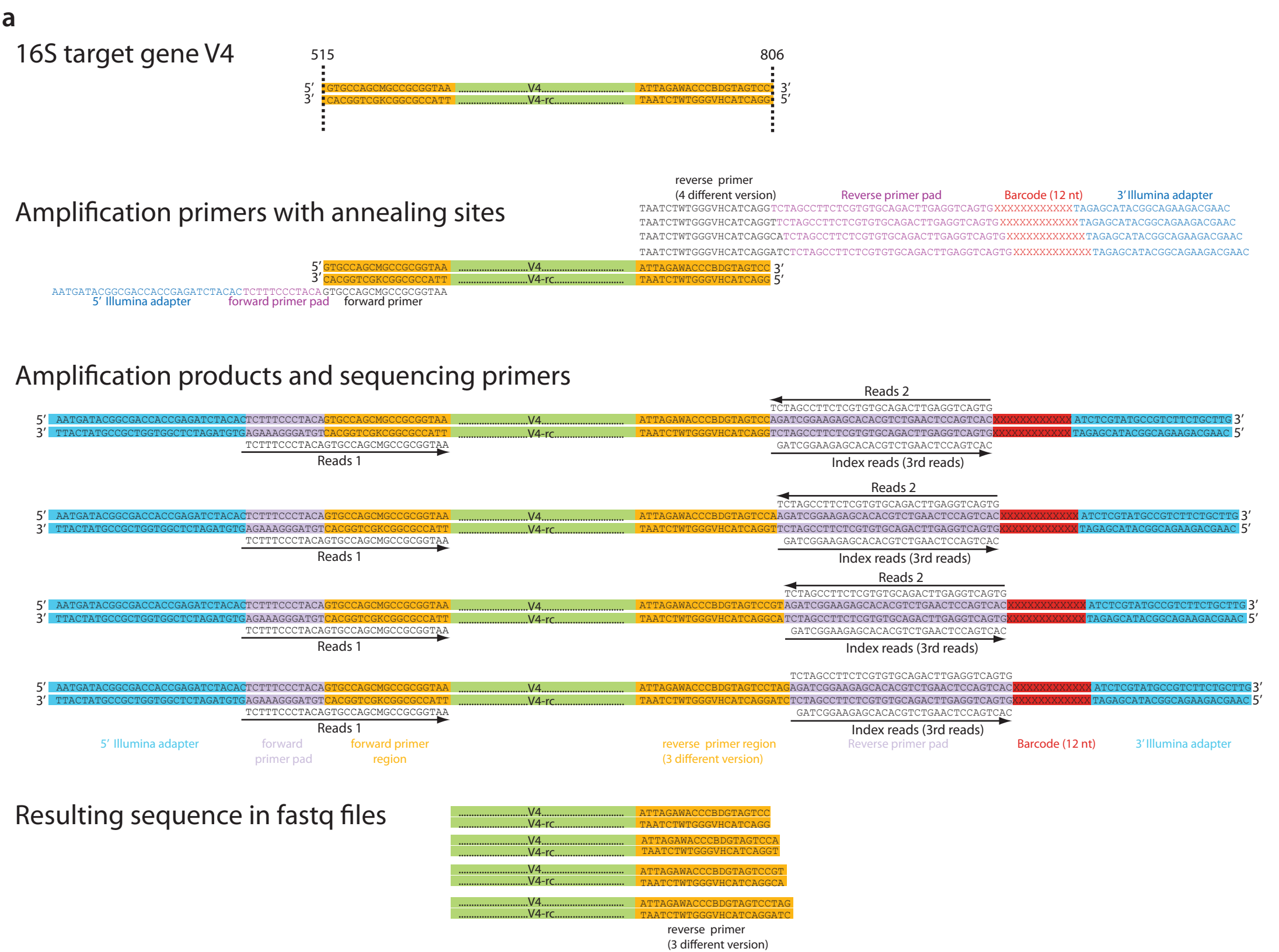
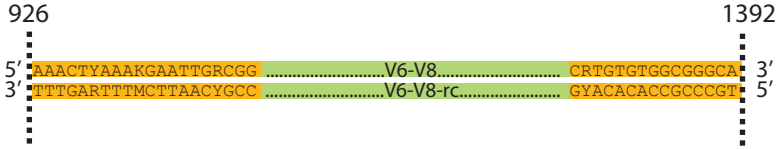
a

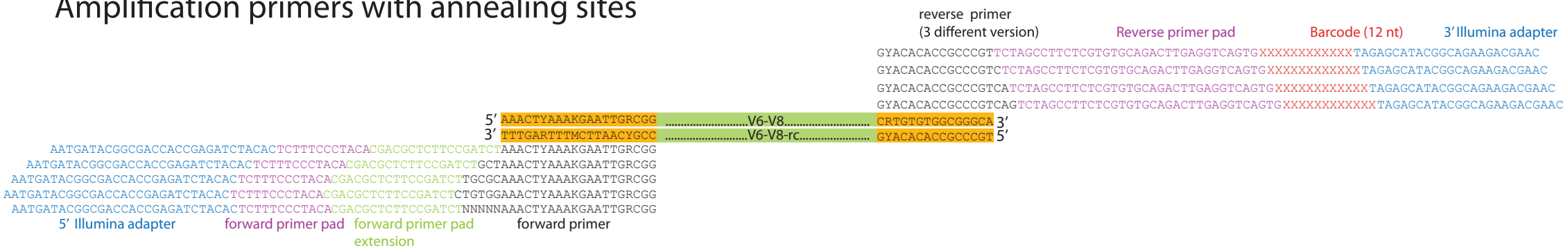

16S target gene V4
515
806
5′ GTGCCAGCMGCCGCGGTAA ..... V4 ..... ATTAGAWACCCBDGTAGTCC 3′
3′ CACGGTCGKCGGCGCCATT ..... V4-rc ..... TAATCTWTGGGVHCATCAGG 5′
Amplification primers with annealing sites
reverse primer
(4 different version)
Reverse primer pad
Barcode (12 nt)
3′ Illumina adapter
TAATCTWTGGGVHCATCAGGTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC
TAATCTWTGGGVHCATCAGGTTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC
TAATCTWTGGGVHCATCAGGCATCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC
TAATCTWTGGGVHCATCAGGATCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC
5′ GTGCCAGCMGCCGCGGTAA ..... V4 ..... ATTAGAWACCCBDGTAGTCC 3′
3′ CACGGTCGKCGGCGCCATT ..... V4-rc ..... TAATCTWTGGGVHCATCAGG 5′
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGTGCCAGCMGCCGCGGTAA
5′ Illumina adapter
forward primer pad
forward primer
Amplification products and sequencing primers
Reads 2
TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGTGCCAGCMGCCGCGGTAA ..... V4 ..... ATTAGAWACCCBDGTAGTCCAGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3′
3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTCACGGTCGKCGGCGCCATT ..... V4-rc ..... TAATCTWTGGGVHCATCAGGTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5′
TCTTTCCCTACAGTGCCAGCMGCCGCGGTAA
Reads 1
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Index reads (3rd reads)
Reads 2
TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGTGCCAGCMGCCGCGGTAA ..... V4 ..... ATTAGAWACCCBDGTAGTCCAAGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3′
3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTCACGGTCGKCGGCGCCATT ..... V4-rc ..... TAATCTWTGGGVHCATCAGGTTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5′
TCTTTCCCTACAGTGCCAGCMGCCGCGGTAA
Reads 1
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Index reads (3rd reads)
Reads 2
TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGTGCCAGCMGCCGCGGTAA ..... V4 ..... ATTAGAWACCCBDGTAGTCCGTAGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3′
3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTCACGGTCGKCGGCGCCATT ..... V4-rc ..... TAATCTWTGGGVHCATCAGGCATCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5′
TCTTTCCCTACAGTGCCAGCMGCCGCGGTAA
Reads 1
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Index reads (3rd reads)
TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGTGCCAGCMGCCGCGGTAA ..... V4 ..... ATTAGAWACCCBDGTAGTCCTAGAGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3′
3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTCACGGTCGKCGGCGCCATT ..... V4-rc ..... TAATCTWTGGGVHCATCAGGATCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5′
TCTTTCCCTACAGTGCCAGCMGCCGCGGTAA
Reads 1
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Index reads (3rd reads)
5′ Illumina adapter
forward primer pad
forward primer region
reverse primer region
(3 different version)
Reverse primer pad
Barcode (12 nt)
3′ Illumina adapter
Resulting sequence in fastq files
..... V4 ..... ATTAGAWACCCBDGTAGTCC
..... V4-rc ..... TAATCTWTGGGVHCATCAGG
..... V4 ..... ATTAGAWACCCBDGTAGTCCA
..... V4-rc ..... TAATCTWTGGGVHCATCAGGT
..... V4 ..... ATTAGAWACCCBDGTAGTCCGT
..... V4-rc ..... TAATCTWTGGGVHCATCAGGCA
..... V4 ..... ATTAGAWACCCBDGTAGTCCTAG
..... V4-rc ..... TAATCTWTGGGVHCATCAGGATC
reverse primer
(3 different version)

b

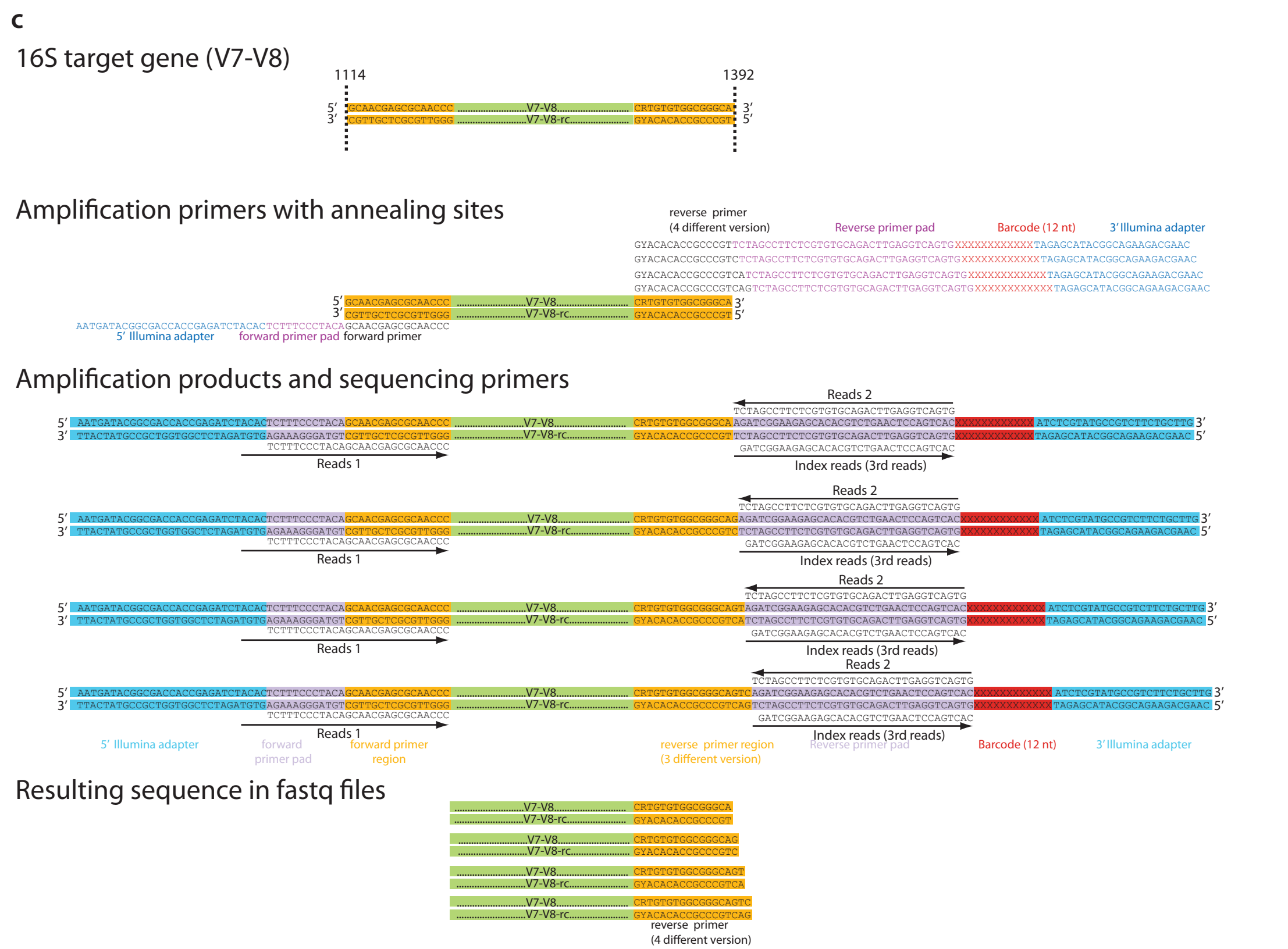
16S target gene (V6-V8)

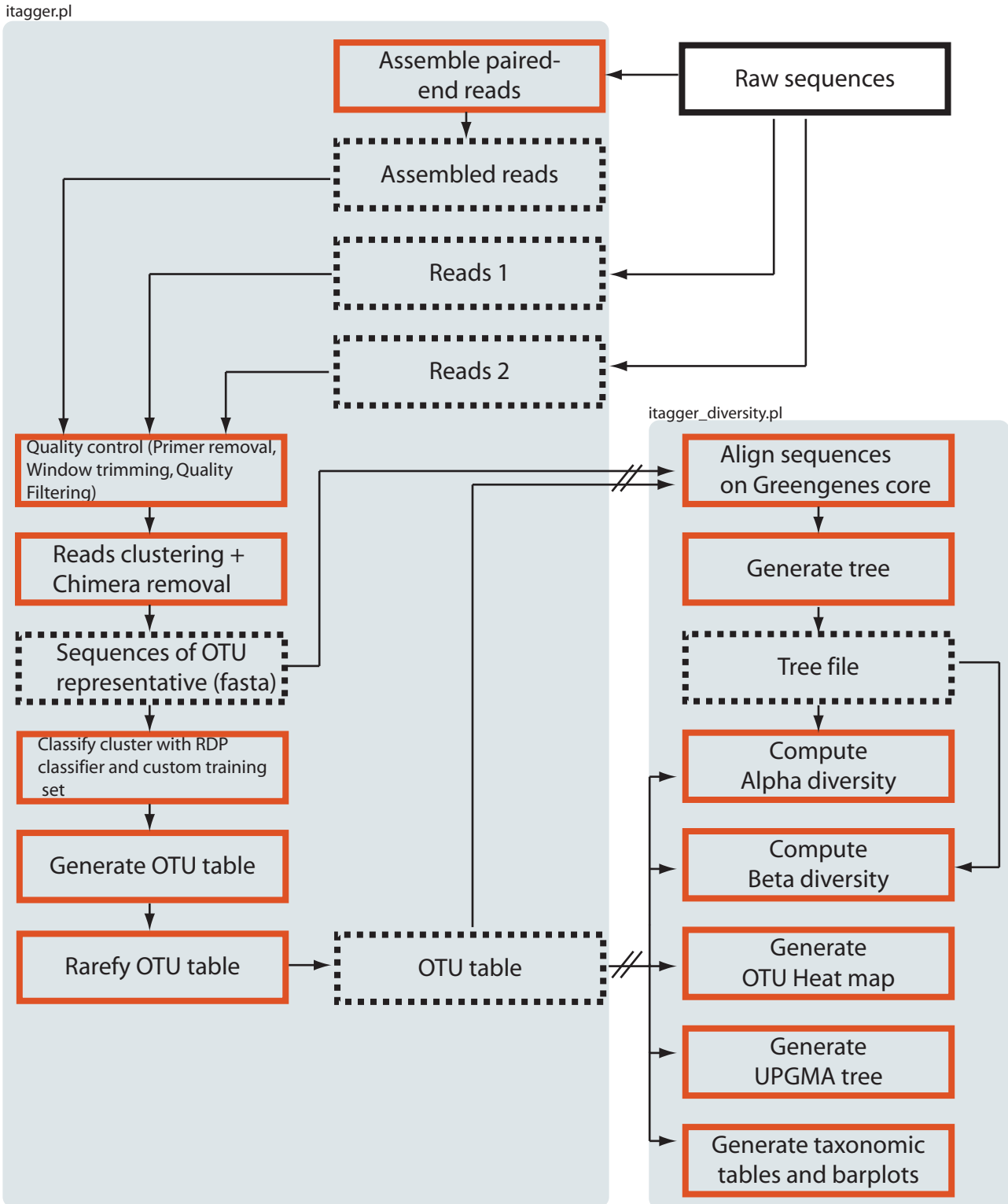


Amplification primers with annealing sites



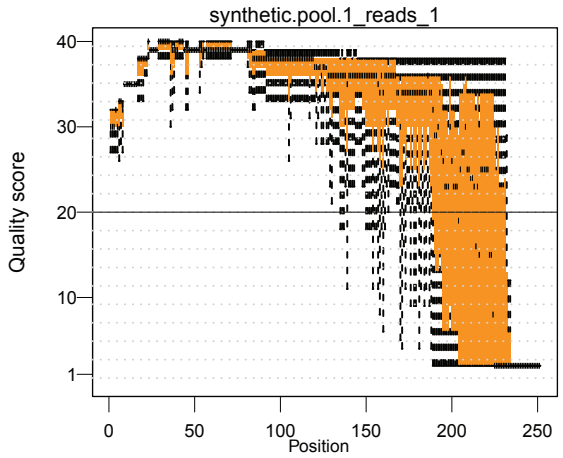
c

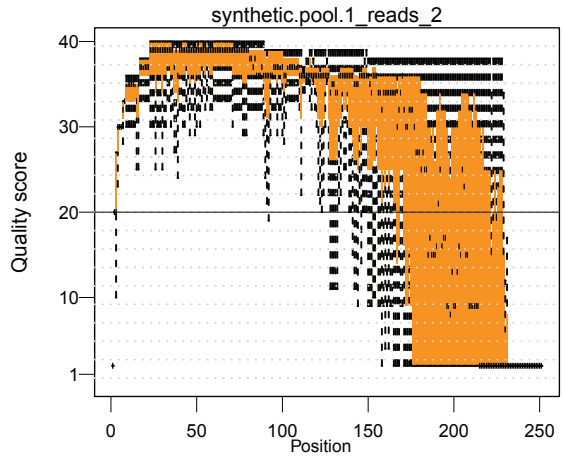

16S target gene (V7-V8)
1114
1392
5′ GCAACGAGCGCAACCC .....V7-V8..... CRTGTGTGGCGGGCA 3′
3′ CGTTGCTCGCGTTGGG .....V7-V8-rc..... GYACACACCGCCCGT 5′
Amplification primers with annealing sites
reverse primer
(4 different version)
Reverse primer pad
Barcode (12 nt)
3′ Illumina adapter
GYACACACCGCCCGTTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC
GYACACACCGCCCGTCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC
GYACACACCGCCCGTCATCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC
GYACACACCGCCCGTCAGTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC
5′ GCAACGAGCGCAACCC .....V7-V8..... CRTGTGTGGCGGGCA 3′
3′ CGTTGCTCGCGTTGGG .....V7-V8-rc..... GYACACACCGCCCGT 5′
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGCAACGAGCGCAACCC
5′ Illumina adapter
forward primer pad
forward primer
Amplification products and sequencing primers
Reads 2
TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGCAACGAGCGCAACCC .....V7-V8..... CRTGTGTGGCGGGCAAGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3′
3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTCGTTGCTCGCGTTGGG .....V7-V8-rc..... GYACACACCGCCCGTCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5′
TCTTTCCCTACAGCAACGAGCGCAACCC
Reads 1
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Index reads (3rd reads)
Reads 2
TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGCAACGAGCGCAACCC .....V7-V8..... CRTGTGTGGCGGGCAGAGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3′
3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTCGTTGCTCGCGTTGGG .....V7-V8-rc..... GYACACACCGCCCGTCTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5′
TCTTTCCCTACAGCAACGAGCGCAACCC
Reads 1
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Index reads (3rd reads)
Reads 2
TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGCAACGAGCGCAACCC .....V7-V8..... CRTGTGTGGCGGGCAGTAGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3′
3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTCGTTGCTCGCGTTGGG .....V7-V8-rc..... GYACACACCGCCCGTCATCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5′
TCTTTCCCTACAGCAACGAGCGCAACCC
Reads 1
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Index reads (3rd reads)
Reads 2
TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACAGCAACGAGCGCAACCC .....V7-V8..... CRTGTGTGGCGGGCAGTCAGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3′
3′ TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTCGTTGCTCGCGTTGGG .....V7-V8-rc..... GYACACACCGCCCGTCAGTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXXXXXXXTAGAGCATACGGCAGAAGACGAAC 5′
TCTTTCCCTACAGCAACGAGCGCAACCC
Reads 1
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Index reads (3rd reads)
5′ Illumina adapter
forward primer pad
forward primer region
reverse primer region
(3 different version)
Reverse primer pad
Barcode (12 nt)
3′ Illumina adapter
Resulting sequence in fastq files
.....V7-V8..... CRTGTGTGGCGGGCA
.....V7-V8-rc..... GYACACACCGCCCGT
.....V7-V8..... CRTGTGTGGCGGGCAG
.....V7-V8-rc..... GYACACACCGCCCGTC
.....V7-V8..... CRTGTGTGGCGGGCAGT
.....V7-V8-rc..... GYACACACCGCCCGTCA
.....V7-V8..... CRTGTGTGGCGGGCAGTC
.....V7-V8-rc..... GYACACACCGCCCGTCAG
reverse primer
(4 different version)



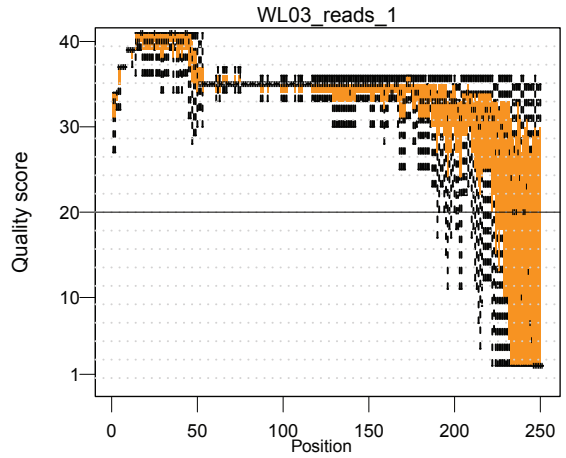
Supplemental figure S2. Itagger pipeline schematics. Dashed lined boxes represent important files generated throughout the pipeline. Analysis pipeline is divided in two main parts. First, raw sequences are processed and clustered to generate an OTU table (itagger.pl). Using multiple OTU tables as input, various diversity metrics are then computed.

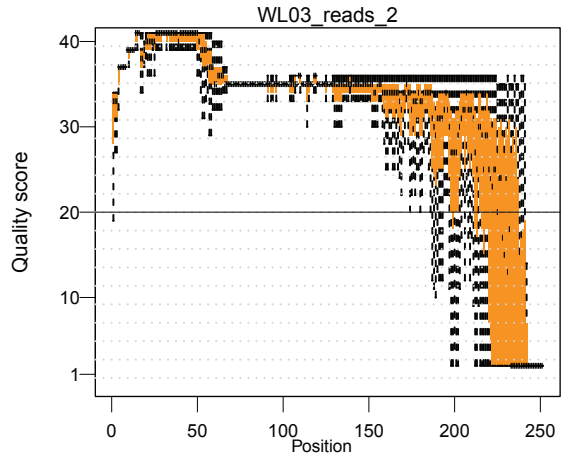
MiSeq V4 P. suwonensis and synthetic community



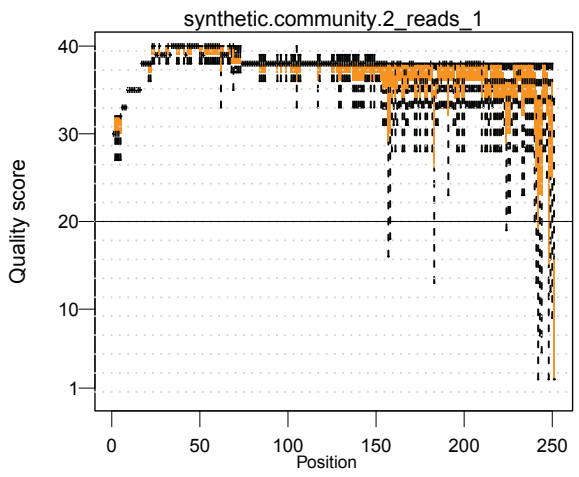


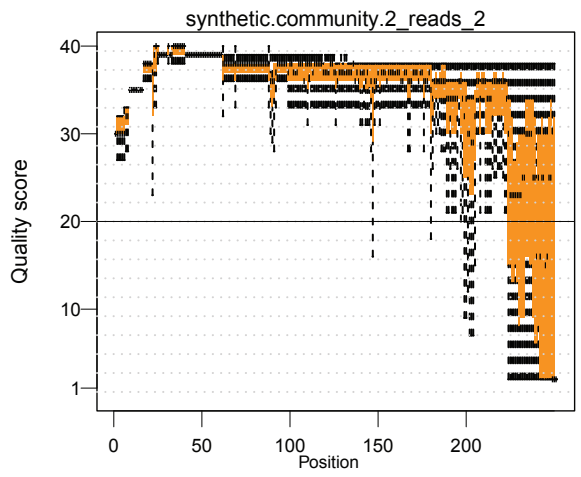
MiSeq V4 wetlands samples



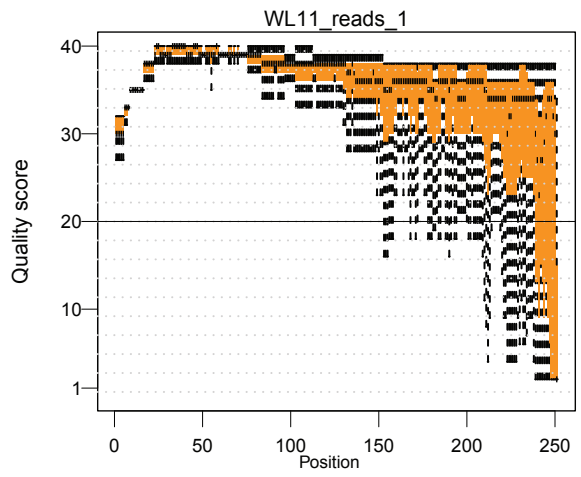


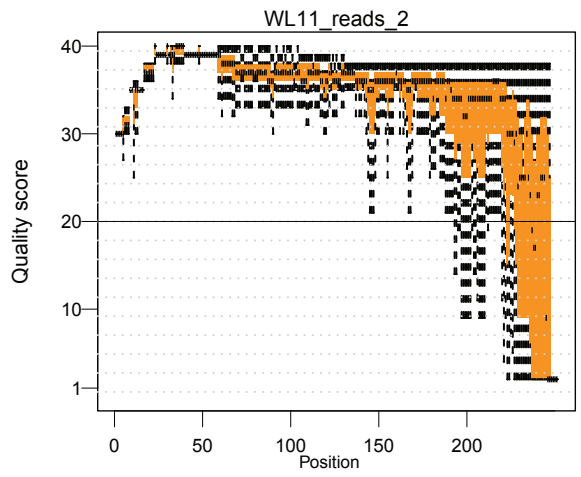
MiSeq V7-V8 P. suwonensis and synthetic community



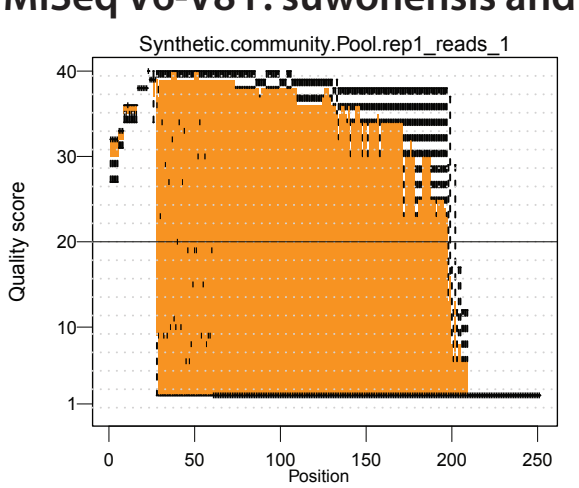


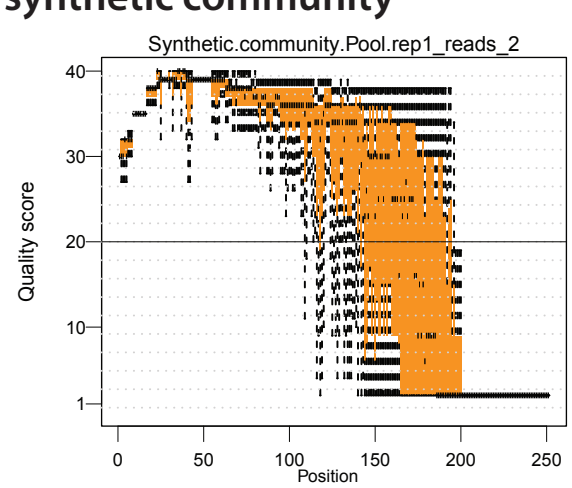
MiSeq V7-V8 wetlands samples



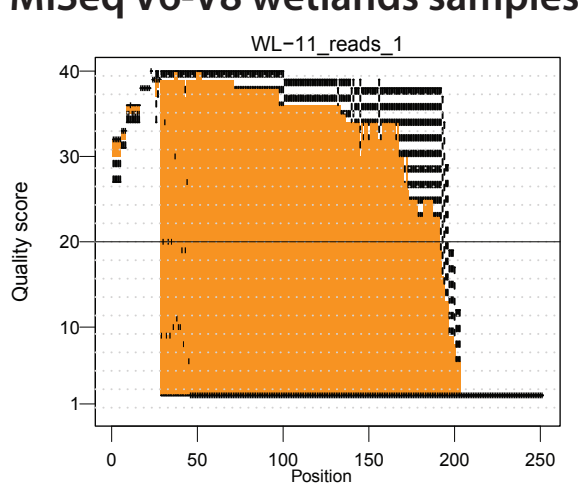


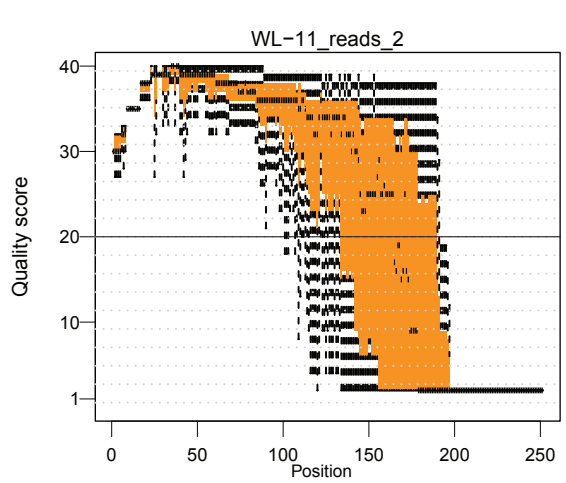
MiSeq V6-V8 P. suwonensis and synthetic community



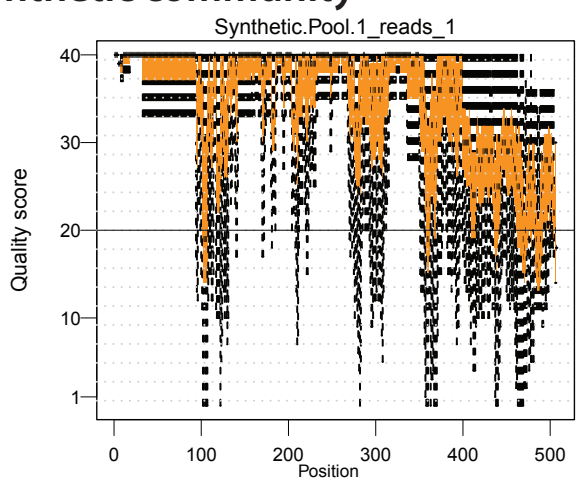


MiSeq V6-V8 wetlands samples





454 V6-V8 P. suwonensis and synthetic community



454 V6-V8 wetlands samples

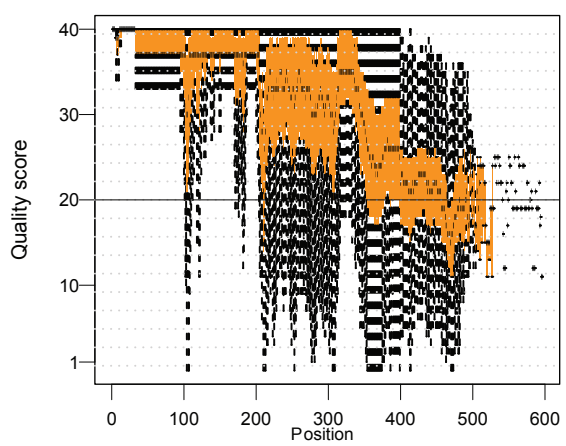
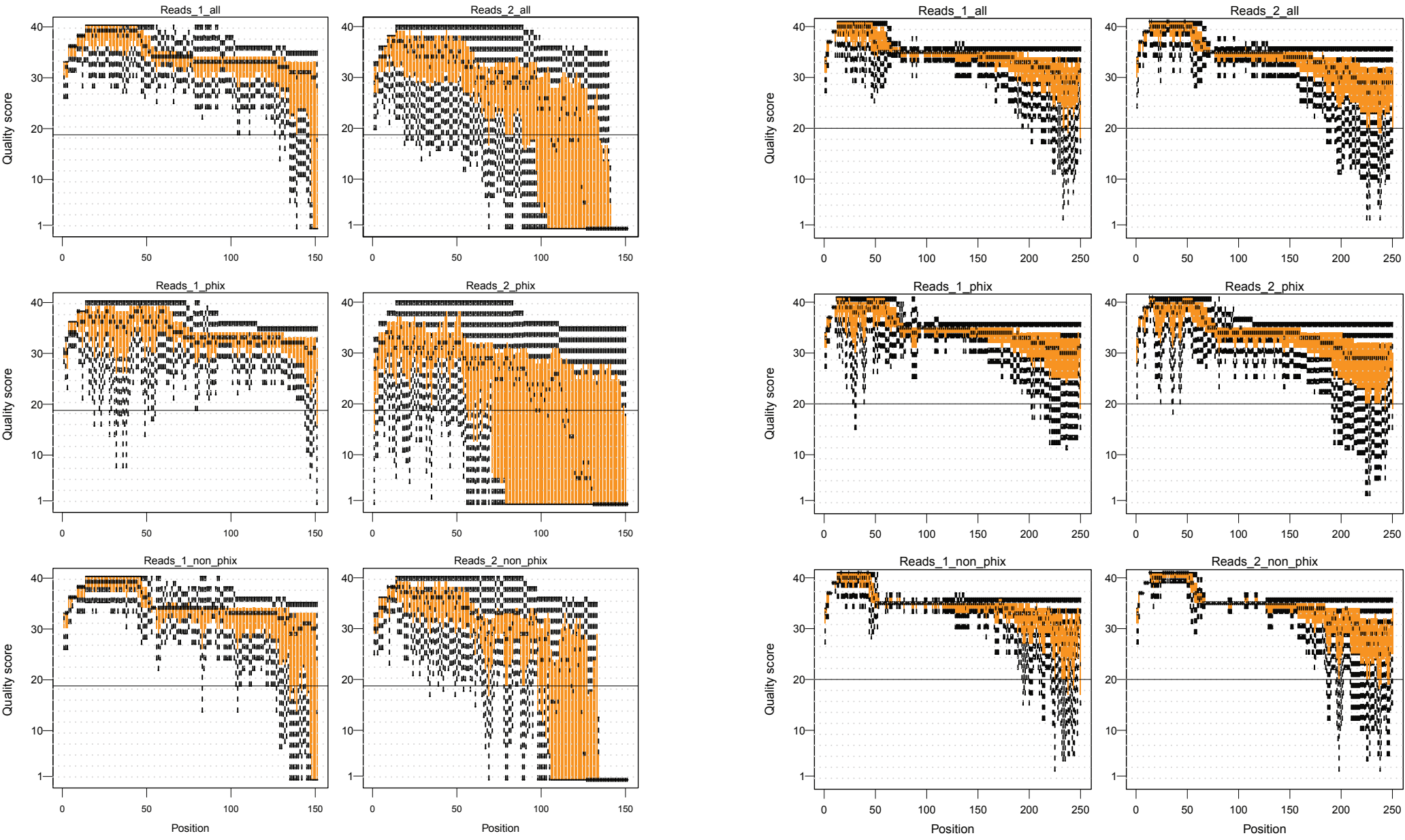


Figure S3. Quality score plots representative for each condition. A meaningful representative quality score plot was chosen among all samples/barcodes for each sequencing condition tested in this study. Orange bars represent the Interquartile range.

a Standard(non-staggered) V4 primers

b Staggered V4 primers



c Typical quality plot of pyrotags obtained with 454 Ti FLX sequencer

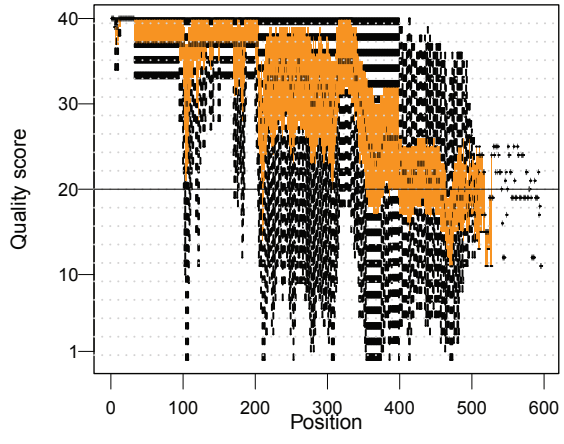


Figure S4. Boxplots of quality score plots for staggered vs non-staggered amplicons for V4 hypervariable region amplified from wetlands samples. Each sequencing run a) staggered and b) non-staggered amplicons) represent a complete MiSeq lane. Quality score plots were generated for all reads, reads 1 and reads 2 and were also split (and plotted) as PhiX and non-PhiX reads. Quality score plots of reads sequenced with 454 Ti FLX are also shown (c). Orange bars represent the Interquartile range.

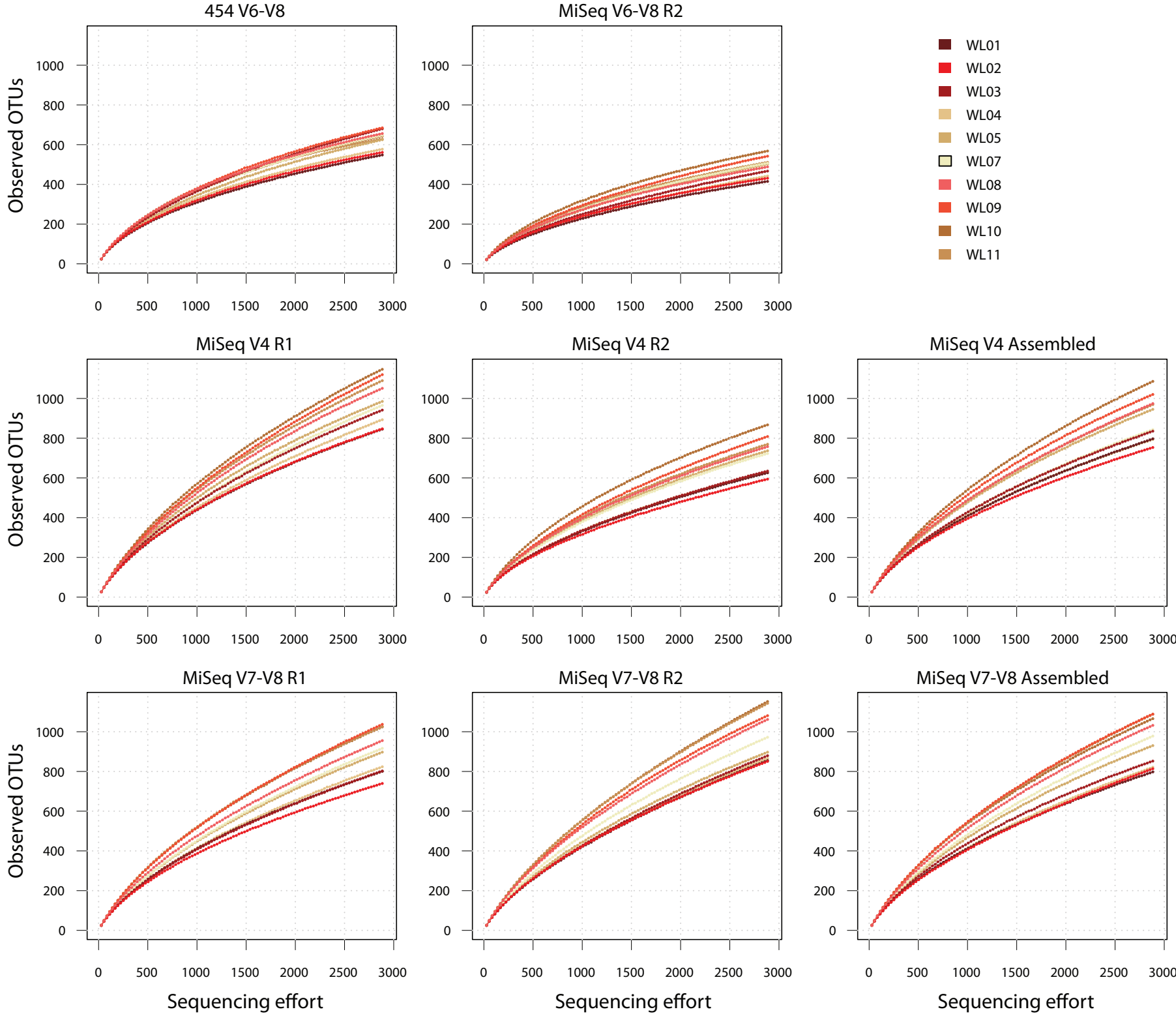


Figure S5. Alpha diversity rarefaction curves (Observed OTUs) for wetlands samples.

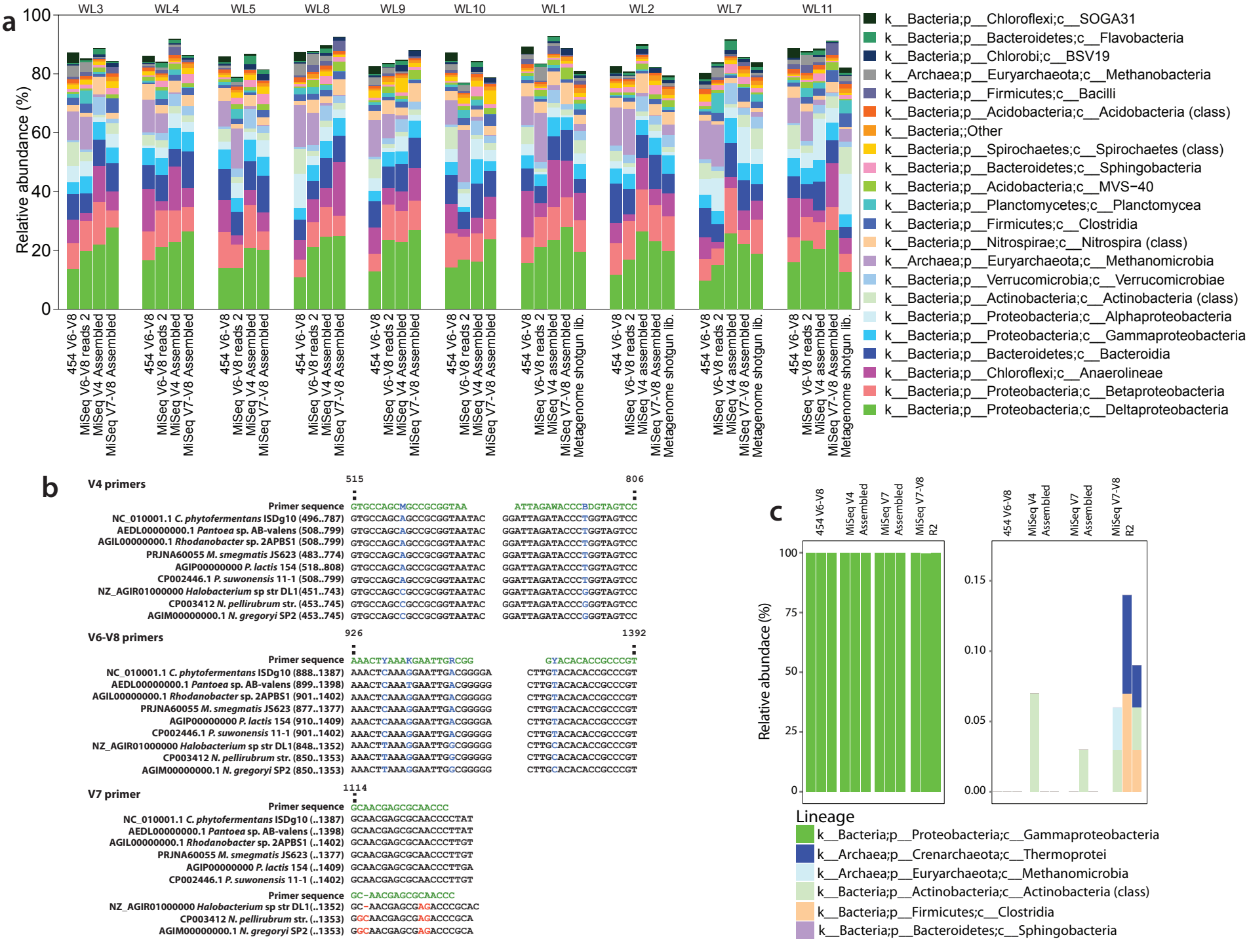


Figure S6. Taxonomic classification at the Class depth for **(a)** samples of a wetlands sampling site. Taxonomy was established for the 22 most abundant OTUs. **b)** Alignments of V4, V6-V7 and V6-V8 primer pairs against synthetic community 16S rRNA annealing regions. Red=mismatch, blue=ambiguous nucleotide match. **c)** Classification of OTUs at the Class depth for samples containing *P. suwonensis* species. For ambiguous nucleotides; M=A or C; B=C or G or T; R=A or G; Y=C or T; K=G or T.

Table S1. Error rate.

	454 V6-V8			MiSeq V4 Assembled			MiSeq V7-V8 Assembled			MiSeq V6-V8 R2		
	Ins.	Del.	Sub.	Ins.	Del.	Sub.	Ins.	Del.	Sub.	Ins.	Del.	Sub.
Average												
No QC	1.55833	0.77000	2.33788	0.00441	0.02501	3.02349	0.00630	0.07551	2.03523	0.00667	3.90917	4.21250
Lenient QC	1.13368	0.57950	2.15685	0.00046	0.01317	1.47008	0.00215	0.05535	1.47008	0.00000	0.09387	1.37669
Stringent QC	0.12272	0.21745	2.20531	0.00086	0.21745	2.20531	0.00086	0.04537	1.35686	0.00000	0.07796	1.13673
Std. Dev.												
No QC	0.60560	0.08239	0.54394	0.00243	0.01466	0.35334	0.00388	0.01053	0.23679	0.00402	1.43616	0.60560
Lenient QC	0.63742	0.08480	0.55671	0.00000	0.00693	0.38657	0.00192	0.01649	0.21710	0.00000	0.05858	0.63742
Stringent QC	0.06759	0.03488	0.64771	0.00031	0.00597	0.34553	0.00067	0.02006	0.16868	0.00000	0.03899	0.06759

For the lenient QC condition, sequences having more than 5 Ns, average quality score lower than 30, or more than 10 nucleotides having a quality score lower than 15 were rejected. The stringent QC condition rejected sequences that had 1 N or more; had average quality scores lower than 33; or had more than 3 nucleotides with a quality score lower than 20. Primers used for amplification where removed *in silico* before quality filtering.

Table S2. 16S reads classification from metagenomic libraries.

	1947.2.1687 (WL01)	**1926.6.1680 (WL02)**	**2004.3.1713 (WL07)**	**1947.3.1687 (WL11)**
Total reads	399,039,592	380,204,200	359,517,560	348,284,236
Contaminants	8,676,422	6,289,485	5,160,841	8,145,940
Non-contaminants	390,363,170	373,914,715	354,356,719	340,138,296
Non-contaminants non-rRNA	346,099,842	331,319,449	307,830,126	299,036,812
Non-contaminants rRNA	44,263,328	42,595,266	46,526,593	41,101,484
Reads used for clustering and/or classification (i.e. after merging, filtering and concatenation)	4,276,356	3,070,676	3,177,536	3,704,636
Reads that classified at least at the kingdom level using bootstrap >= 0.50 (euk and prok)	2,137,295	1,492,987	1,655,595	2,094,822
Reads that classified at least at the kingdom-bacteria/archaea level using RDP threshold >= 0.50	40,210	34,677	36,272	35,586

Table S3. Reads and OTU counts through clustering steps for *P. suwonensis.*

	454 V6-V8	MiSeq V4 Assembled	MiSeq V7-V8 Assembled	MiSeq V6-V8 Reads 2
QCed reads	36,770	60,769	610,836	392,270
100% identity OTUs	3,680	1,943	25,061	3,043
99% identity OTUs	1,038	368	11,072	1,792
99% > 2	217	68	1,451	338
After de novo chimera removal	217	67	1,448	337
After reference chimera removal	217	66	1,438	337
97% OTUs	2	3	10	20

Table S4. Reads and OTU counts through clustering steps for synthetic community.

	454 V6-V8	MiSeq V4 Assembled	MiSeq V7-V8 Assembled	MiSeq V6-V8 Reads 2
QCed reads	57,097	102,283	748,525	413,445
100% identity OTUs	8,928	7,166	35,185	7,670
99% identity OTUs	2,466	1,255	10,730	4,511
99% > 2	483	326	2,079	1,170
After de novo chimera removal	483	220	1,804	1,163
After reference chimera removal	443	216	1,211	1,150
97% OTUs	16	20	32	89

Table S5. Reads and OTU counts through clustering steps for wetlands samples.

	454 V6-V8	MiSeq V4 Assembled	MiSeq V7-V8 Assembled	MiSeq V6-V8 Reads 2
QCed reads	99,897	635,906	2,641,996	1,105,248
100% identity OTUs	59,083	305,841	1,164,152	162,273
99% identity OTUs	21,218	178,204	650,395	109,929
99% > 2	4,323	19,220	62,482	19,778
After de novo chimera removal	4,260	16,947	53,914	19,749
After reference chimera removal	4,189	16,695	49,956	19,589
97% OTUs	1,985	6,901	13,254	4,263