Supplementary Material 1. Full list of primers used. Degenerate primer sequences for standard 515F and 806R primer pools are shown (top), together with level of degeneracy, primer lengths, and calculated melting temperature (Tm) ranges. Degenerate positions are highlighted in red. Truncated primer pools are also shown (tr515F and tr806R). Individual primer sequences are shown for each pool (standard 515F, tr515F, standard 806R, and tr806R).

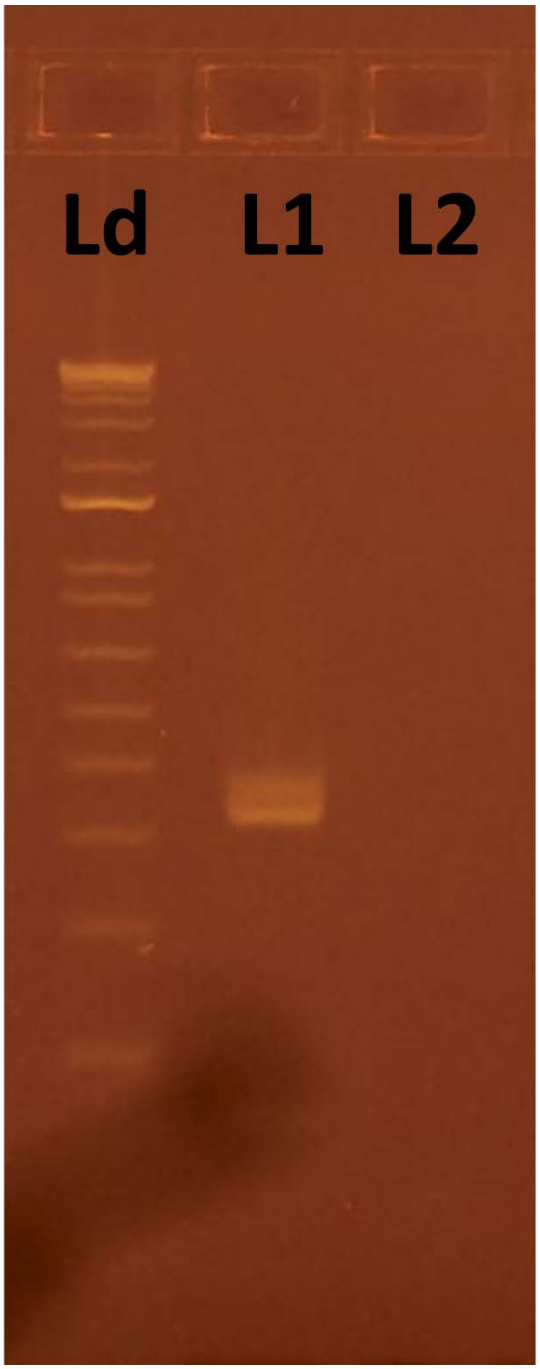
Type	Primer	Degeneracy	Sequence	Length	Tm (°C) Range
Pool	515F	4	GTGYCAGCMGCCGCGGTAA	19	66.9-71.8
Pool	tr515F	8	TGYCAGCMGCCGCGGT	10-12	55.8-58.6
Pool	806R	24	GGACTACNVGGGTWTCTAAT	20	54.5-61.5
Pool	tr806R	28	GGACTACNVGGGTWTCTAAT	13-20	52-56.7

*Blue represents common positions contained in all variants. Yellow represents variable 5' and 3' ends.

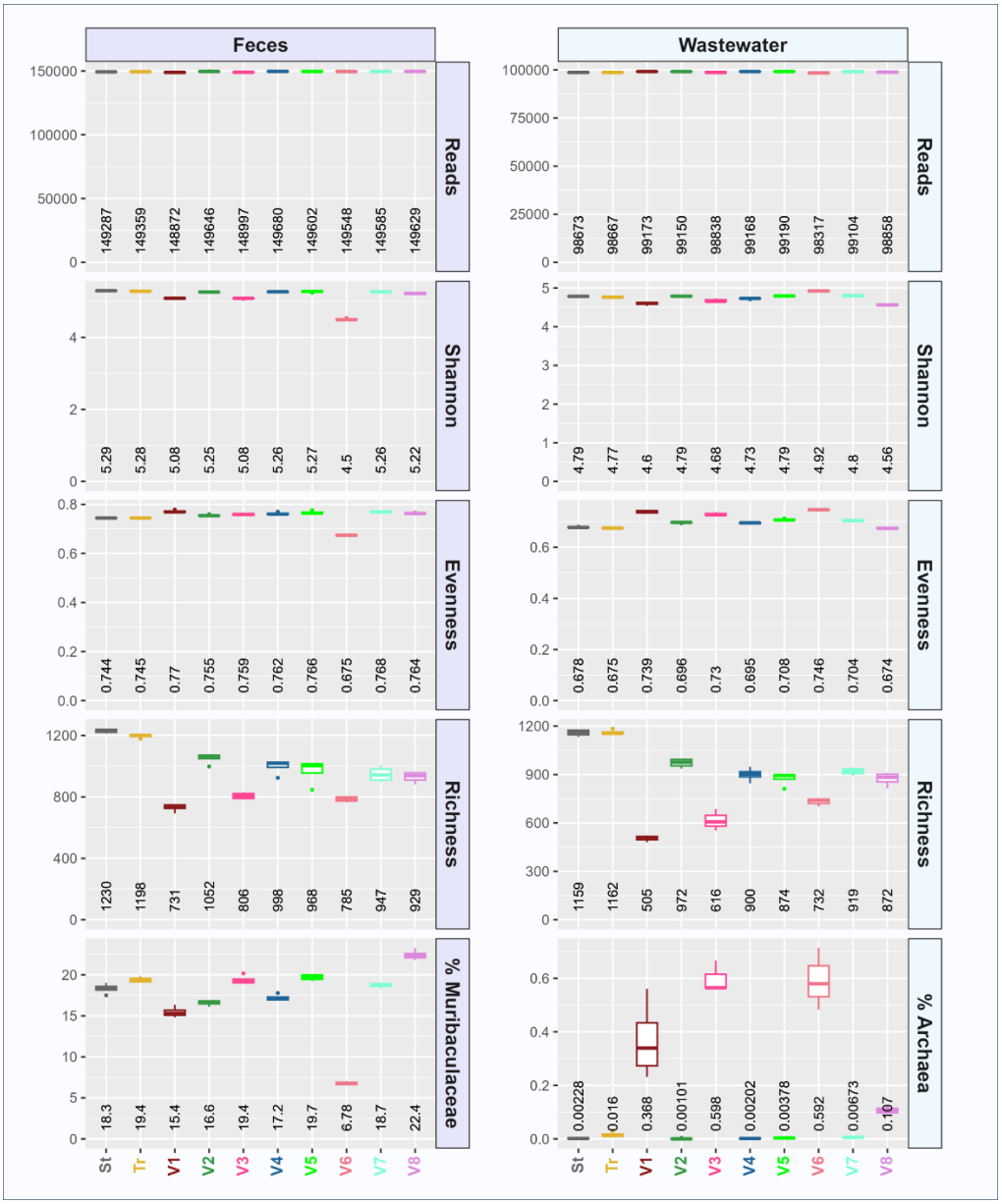
		Degeneracy				Position		
Type	Primer	4th	9th	Sequence	Length	Start	End	Tm (°C)
Individual Standard Forward	515F-1	C	A	GTGCCAGCAGCCGCGGTAA	19	1	19	69.6
	515F-2	C	C	GTGCCAGCCGCCGCGGTAA	19	1	19	71.8
	515F-3	T	A	GTGTCAGCAGCCGCGGTAA	19	1	19	66.9
	515F-4	T	C	GTGTCAGCCGCCGCGGTAA	19	1	19	69.1
Individual Truncated Forward	V1-tr515F	C	C	TGCCAGCCGCC	11	2	12	56.6
	V2-tr515F	C	A	TGCCAGCAGCCG	12	2	13	57.2
	V3-tr515F	T	C	GTCAGCCGCCGC	12	3	14	58.6
	V4-tr515F	T	A	TCAGCAGCCGCG	12	4	15	57.2
	V5-tr515F	-	A	CAGCAGCCGCGG	12	5	16	58.6
	V6-tr515F	-	C	AGCCGCCGCG	10	6	15	56.4
	V7-tr515F	-	A	GCAGCCGCGGT	11	7	17	57.1
	V8-tr515F	-	C	CCGCCGCGGT	10	8	17	55.8
	Primer	8th	9th	14th Sequence	Length	Start	End	Tm (°C)
Individual Standard Reverse	806R-1	A	A	A GGACTACAAGGGTATCTAAT	20	1	20	55.7
	806R-2	A	A	T GGACTACAAGGGTTTCTAAT	20	1	20	56.7
	806R-3	A	C	A GGACTACACGGGTATCTAAT	20	1	20	58
	806R-4	A	C	T GGACTACACGGGTTTCTAAT	20	1	20	59
	806R-5	A	G	A GGACTACAGGGGTATCTAAT	20	1	20	57.4
	806R-6	A	G	T GGACTACAGGGGTTTCTAAT	20	1	20	58.5
	806R-7	C	A	A GGACTACCAGGGTATCTAAT	20	1	20	57.4
	806R-8	C	A	T GGACTACCAGGGTTTCTAAT	20	1	20	58.5
	806R-9	C	C	A GGACTACCCGGGTATCTAAT	20	1	20	59.8
	806R-10	C	C	T GGACTACCCGGGTTTCTAAT	20	1	20	60.8
	806R-11	C	G	A GGACTACCGGGGTATCTAAT	20	1	20	59.8
	806R-12	C	G	T GGACTACCGGGGTTTCTAAT	20	1	20	60.8
	806R-13	G	A	A GGACTACGAGGGTATCTAAT	20	1	20	57.4
	806R-14	G	A	T GGACTACGAGGGTTTCTAAT	20	1	20	58.4
	806R-15	G	C	A GGACTACGCGGGTATCTAAT	20	1	20	60.5
	806R-16	G	C	T GGACTACGCGGGTTTCTAAT	20	1	20	61.5
	806R-17	G	G	A GGACTACGGGGGTATCTAAT	20	1	20	59.8
	806R-18	G	G	T GGACTACGGGGGTTTCTAAT	20	1	20	60.8
	806R-19	T	A	A GGACTACTAGGGTATCTAAT	20	1	20	54.5
	806R-20	T	A	T GGACTACTAGGGTTTCTAAT	20	1	20	55.6
	806R-21	T	C	A GGACTACTCGGGTATCTAAT	20	1	20	57.4
	806R-22	T	C	T GGACTACTCGGGTTTCTAAT	20	1	20	58.4
	806R-23	T	G	A GGACTACTGGGGTATCTAAT	20	1	20	57.4
	806R-24	T	G	T GGACTACTGGGGTTTCTAAT	20	1	20	58.5
Individual Truncated Reverse	V1-tr806R	A	C	A GGACTACACGGGTAT	15	1	15	52.8
	V2-tr806R	A	C	T GGACTACACGGGTTT	15	1	15	54.2
	V3-tr806R	A	G	A GGACTACAGGGGTAT	15	1	15	52
	V4-tr806R	A	G	T GGACTACAGGGGTTT	15	1	15	53.4
	V5-tr806R	T	C	A GGACTACTCGGGTAT	15	1	15	52
	V6-tr806R	T	C	T GGACTACTCGGGTTT	15	1	15	53.5
	V7-tr806R	T	G	A GGACTACTGGGGTAT	15	1	15	52
	V8-tr806R	T	G	T GGACTACTGGGGTTT	15	1	15	53.4
	V9-tr806R	C	A	A GGACTACCAGGGTAT	15	1	15	52
	V10-tr806R	C	A	T GGACTACCAGGGTTT	15	1	15	53.4
	V11-tr806R	G	A	A GGACTACGAGGGTAT	15	1	15	52
	V12-tr806R	G	A	T GGACTACGAGGGTTT	15	1	15	53.5
	V13-tr806R	C	C	- GGACTACCCGGGT	13	1	13	54.3
	V14-tr806R	C	G	- GGACTACCGGGGT	13	1	13	54.3
	V15-tr806R	G	C	- GGACTACGCGGGT	13	1	13	55.7
	V16-tr806R	G	G	- GGACTACGGGGGT	13	1	13	54.3
	V17-tr806R	C	C	A CTACCCGGGTATCTAA	16	4	19	52.6
	V18-tr806R	C	C	T CTACCCGGGTTTCTAA	16	4	19	54
	V19-tr806R	C	G	A CTACCGGGGTATCTAA	16	4	19	52.6
	V20-tr806R	C	G	T CTACCGGGGTTTCTAA	16	4	19	54
	V21-tr806R	G	C	A CTACGCGGGTATCTAA	16	4	19	53.8
	V22-tr806R	G	C	T CTACGCGGGTTTCTAA	16	4	19	55.1
	V23-tr806R	G	G	A CTACGGGGGTATCTAA	16	4	19	52.6
	V24-tr806R	G	G	T CTACGGGGGTTTCTAA	16	4	19	54
	V25-tr806R	A	A	A GGACTACAAGGGTATCTAAT	20	1	20	55.7
	V26-tr806R	A	A	T GGACTACAAGGGTTTCTAAT	20	1	20	56.7
	V27-tr806R	T	A	A GGACTACTAGGGTATCTAAT	20	1	20	54.5
	V28-tr806R	T	A	T GGACTACTAGGGTTTCTAAT	20	1	20	55.6

*Blue represents common positions contained in all variants. Yellow represents variable 5' and 3' ends.

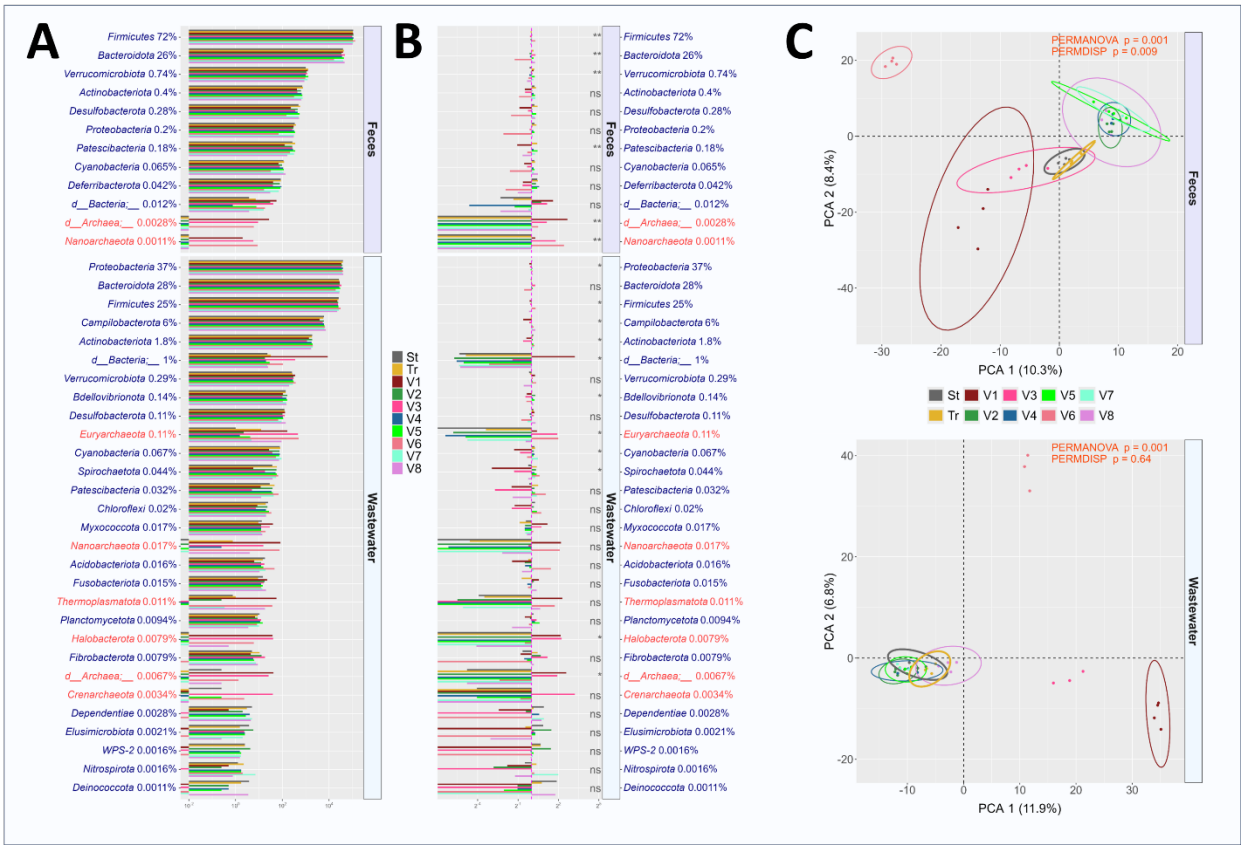
Supplementary Material 2. Amplification of microbial genomic DNA with an 8-base truncated 515F primer with and without 5' linkers. Amplification of the region of interest was achieved when the primer was synthesized with a 5' IDT linker (CTACACGACGCTCTTCCGATCT***GCCGCGGT***; Lane 1; ~340 bp amplicon), while no amplification was achieved in the absence of the 5' linker (***GCCGCGGT***; Lane 2). PCR conditions were identical for both reactions as described in the materials and methods of the main manuscript. Briefly, conditions were an initial denaturation at 98 °C for 30 seconds, followed by 28 cycles of denaturation at 98 °C for 10 seconds, annealing at 52 °C for 5 seconds, and elongation at 68 °C for 1 second. Ld = E-Gel 1 Kb Plus DNA Ladder (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA).



Supplementary Material 3. Alpha diversity metrics for feces and wastewater microbiomes as assessed using PCR with non-degenerate truncated forward primer variants. Feces (left, purple) and wastewater (right, blue) composite DNA samples were PCR amplified using both standard (gray) and truncated (gold) primer pools, in addition to individual 515F truncated primers (V1-V8; various colors). All reactions used the standard 806R primer. Data were rarefied (150,000 and 100,000 sequences per replicate for feces and wastewater, respectively) and filtered by sample type to remove features with a minimum relative abundance of $1/10^5$. Alpha diversity metrics for the observed microbial communities were calculated from ASV level data. The relative abundance of a single taxon relevant to each sample type is also shown (i.e., Muribaculaceae, common murine gut microorganisms for feces; Archaea for wastewater). In wastewater, primer variants V1, V3, V6, and V8 enrich for Archaea, though not to the same level as in soil, leading to reduced alpha diversity indices. In feces, primer variants V1, V3, and V6 yielded lower overall ASV richness, and variant V8 yielded elevated relative abundance of Muribaculaceae.



Supplementary Material 4. Effect of microbiome profiling using standard (515F/806R), truncated (tr515F/806R), and non-degenerate tr515F primer variants on observed microbial community structure. Feces (top, purple) and wastewater (bottom, blue) composite DNA samples were PCR amplified using both standard (515F) and truncated (tr515F) primer pools, in addition to individual 515F truncated primers (V1 through V8). All reactions used the standard 806R primer. Data were rarefied (150,000 and 100,000 sequences per replicate for feces and wastewater, respectively) and filtered by sample type to remove features with a minimum relative abundance of $1/10^5$. (A) Average relative abundance of microbial phyla as measured using standard (gray) or truncated (gold) forward primer pools or eight truncated forward primers (V1 through V8), plotted on log10-scale. (B) Average abundance of each phylum and primer set relative to the mean abundance of each phylum, plotted on log2-scale. To determine the statistical significance of difference between the standard and truncated primer sets, the relative abundances for each phylum were transformed using the centered log-ratio method and compared using a Kruskal-Wallis test, adjusted by the Benjamini-Hochberg method. Significance (q-values <0.05) is indicated by an asterisk. Phyla from the domain Archaea are highlighted in red. (C) Ordination using principal component analysis (PCA) illustrating differences in microbial community structure between samples. In feces (top; F=127.2, p=0.001) and wastewater (bottom; F=416.4.4, p=0.001), observed microbial communities were significantly different as evaluated using PERMANOVA. Replicates from the truncated primer set had, in some cases, greater variability relative to replicates from the standard primer set, and differences in dispersion were not significant (F=0.791, p=0.623) for wastewater, and significant (F=2.758, p=0.001) for feces as evaluated using PERMDISP. In feces, archaeal-enriched truncated primers yielded distinct microbial communities (V1 and V3), though V6 and V8 archaeal-enriched variants were less strongly separated than observed for soil. In wastewater, microbial community profiles generated with V1, V3, and V6 forward primer variants were strongly separated from all other profiles, primarily on the PCA1 axis.


A
B
C
Feces
Wastewater
Firmicutes 72%
Bacteroidota 26%
Verrucomicrobiota 0.74%
Actinobacteriota 0.4%
Desulfobacterota 0.28%
Proteobacteria 0.2%
Patescibacteria 0.18%
Cyanobacteria 0.065%
Deferribacterota 0.042%
d__Bacteria;__ 0.012%
d__Archaea;__ 0.0028%
Nanoarchaeota 0.0011%
Proteobacteria 37%
Bacteroidota 28%
Firmicutes 25%
Campilobacterota 6%
Actinobacteriota 1.8%
d__Bacteria;__ 1%
Verrucomicrobiota 0.29%
Bdellovibrionota 0.14%
Desulfobacterota 0.11%
Euryarchaeota 0.11%
Cyanobacteria 0.067%
Spirochaetota 0.044%
Patescibacteria 0.032%
Chloroflexi 0.02%
Myxococcota 0.017%
Nanoarchaeota 0.017%
Acidobacteriota 0.016%
Fusobacteriota 0.015%
Thermoplasmatota 0.011%
Planctomycetota 0.0094%
Halobacterota 0.0079%
Fibrobacterota 0.0079%
d__Archaea;__ 0.0067%
Crenarchaeota 0.0034%
Dependentiae 0.0028%
Elusimicrobiota 0.0021%
WPS-2 0.0016%
Nitrospirota 0.0016%
Deinococcota 0.0011%
St
Tr
V1
V2
V3
V4
V5
V6
V7
V8
PERMANOVA p = 0.001
PERMDISP p = 0.009
PCA 2 (8.4%)
PCA 1 (10.3%)
PERMANOVA p = 0.001
PERMDISP p = 0.64
PCA 2 (6.8%)
PCA 1 (11.9%)