Supplemental Material

Article title: Host specificity patterns of genus *Bacteroides* human fecal markers across 16S ribosomal RNA gene variable regions and development of qPCR assays targeting a sewage-derived *Bacteroides*

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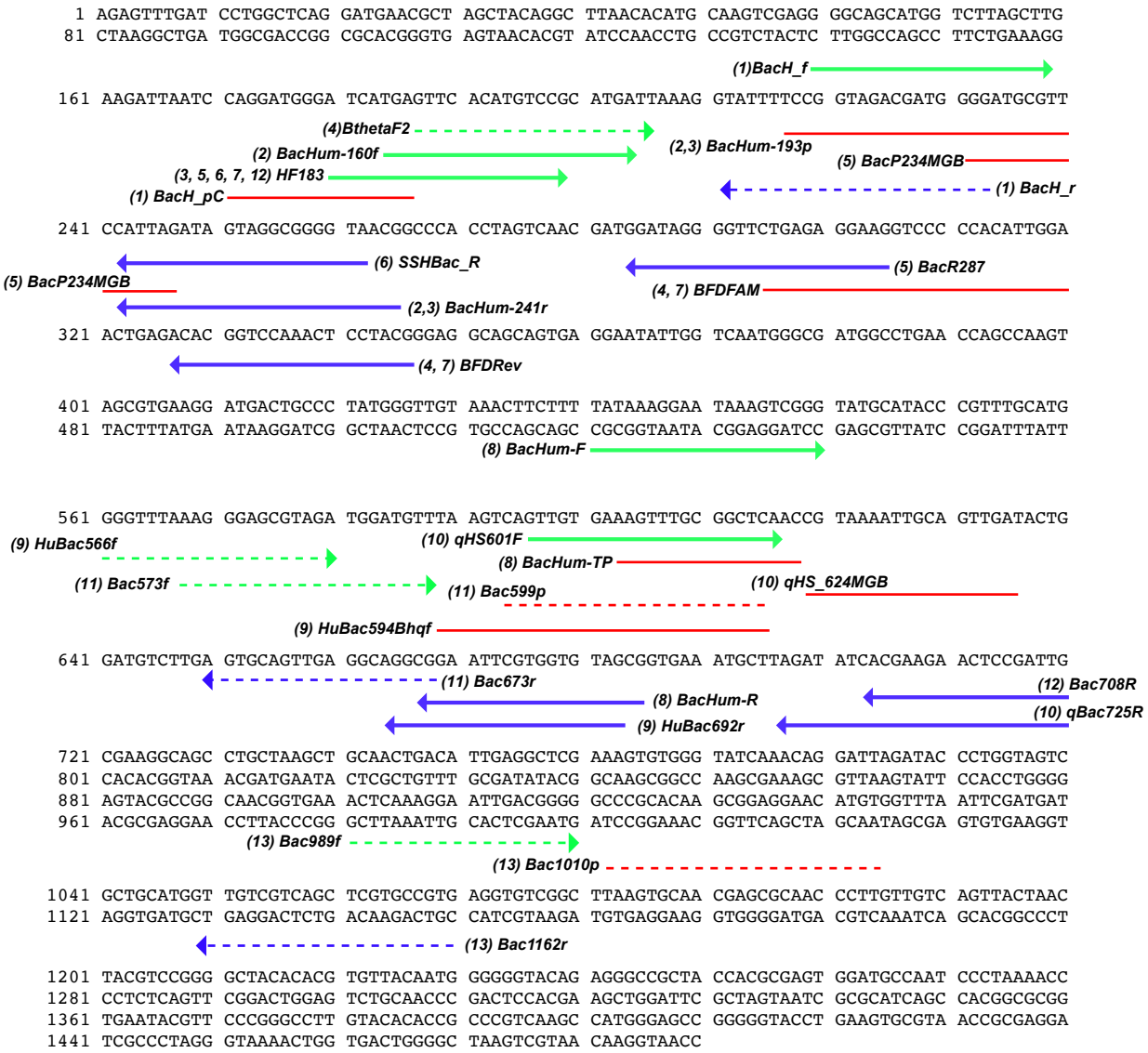

Fig. S1. Alignment of established 16S rRNA gene Bacteroides assays. Primers and probes sequences are aligned to reference sequence (GenBank accession number AB242143). Forward primers are shown in green arrows, probes are shown in red lines, and reverse primers are shown in blue arrows. Sequences that are not 100% matched with the reference are shown in dashed line. Each primer/probe name is labeled at the start or end of the seuqnece. Numbers in parentheses represent the following assays: (1) BacH (Reischer et al. 2006), (2) BacHum-UCD (Kildare et al. 2007), (3) HB (Templar et al. 2016), (4) BthetaF2 (Haugland et al. 2010), (5) HF183/BacR287 (Green et al. 2014), (6) HF183/SSHBac_R (Seurinck et al. 2005), (7) HF183/BFDrev (Haugland et al. 2010), (8) BacHuman (Lee et al. 2010), (9) HuBac (Layton et al. 2006), (10) HumanBac-1 (Okabe et al. 2007), (11) BacV4V5-1, developed by this study, (12) HF183/Bac708R (Bernhard and Field 2000), (13) BacV6-21, developed by this study.

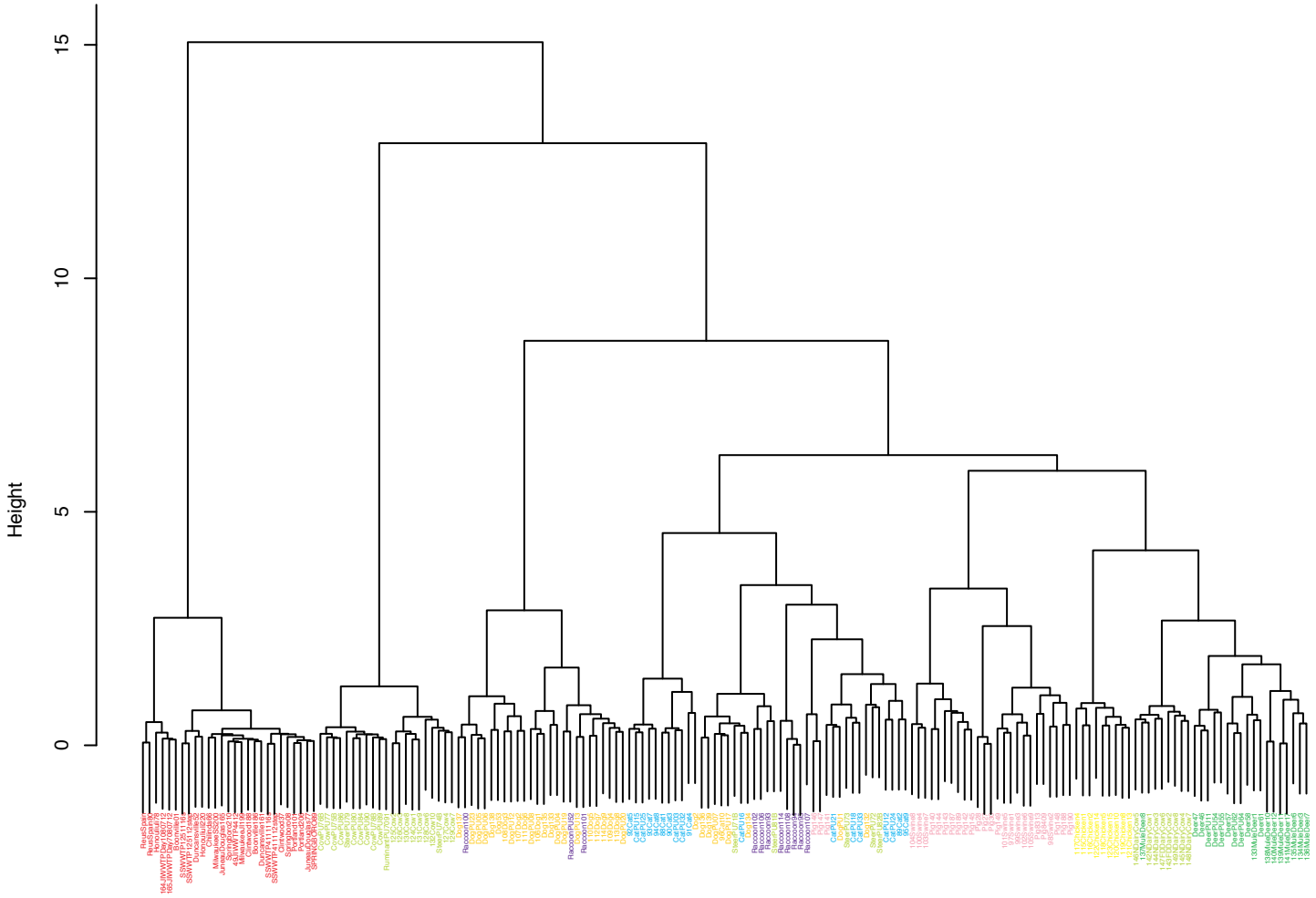

Fig. S2. *Bacteroides* oligotypes hierarchical cluster analysis based on Bray-Curtis dissimilarity. Sewage samples are labeled in red. For animal host labels (left to right): cow samples = light green, dog samples = yellow, raccoon samples = purple, cat samples = blue, pig samples = pink, chicken samples = bright yellow, and deer samples = green.

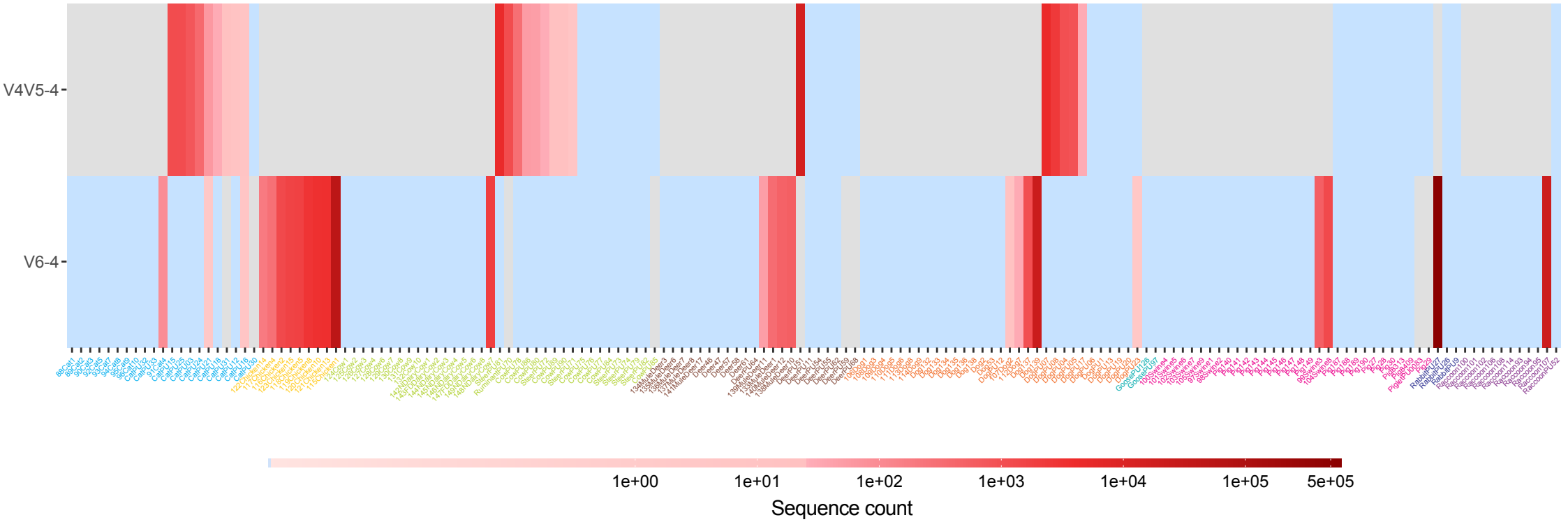

Fig. S3. Host specificity of main types of the HF183 downstream regions (V4V5-4 and V6-4). Gray color = samples are not sequenced. Light blue color = zero sequence count. Positive sequence count values increase from light to dark red. All animals are grouped in host types and labeled in colors as listed below (from left to right): cat samples = blue, chicken samples = yellow, cow samples = light green, deer samples = brown, dog samples = orange, goose samples = green, pig samples = pink, rabbit samples = dark blue, and raccoon samples = purple.

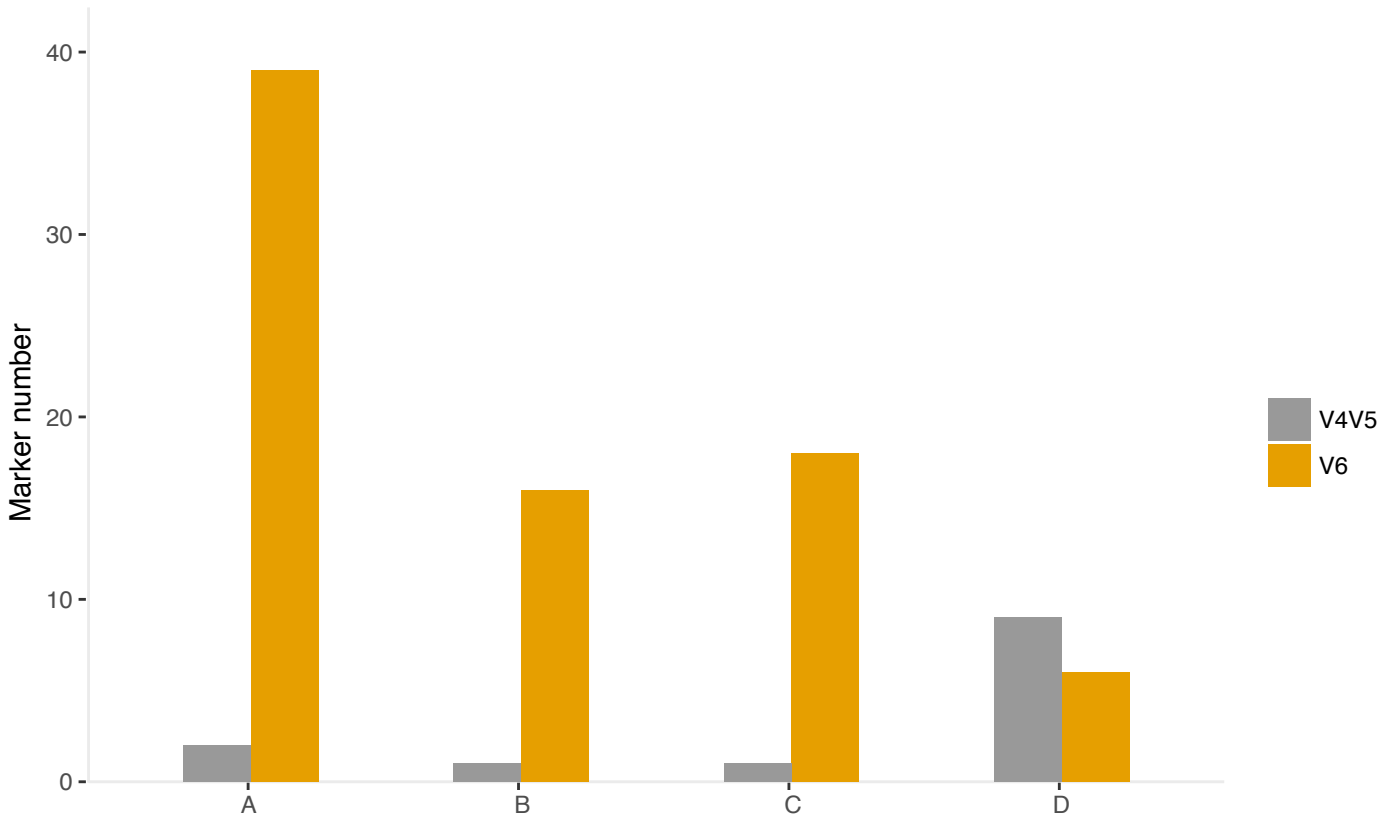

Fig. S4. Comparison of V4V5 and V6 regions for human-associated *Bacteroides* markers identification. Groups of (A) 100% specificity and sensitivity, (B)100% specificity but 90% ~100% sensitivity, (C)100% sensitivity but 90% ~100% specificity, and (D) 90% ~100% specificity and sensitivity are shown. Yellow bars represent V6 region marker numbers and grey bars represent V4V5 region marker numbers.

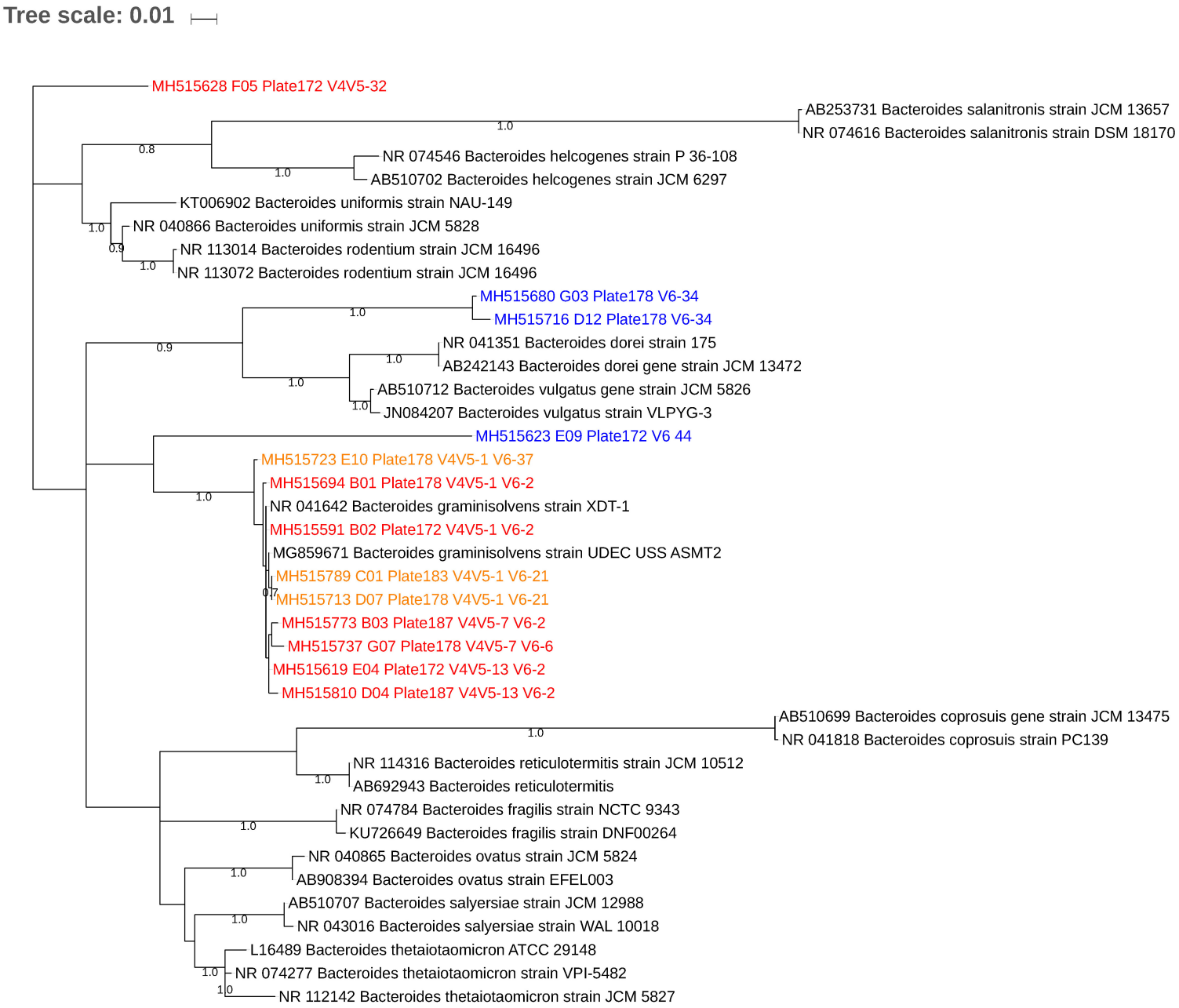

Fig. S5. Maximum likelihood tree constructed from *Bacteroides* reference strains and clones that are found to contain V4V5 and V6 regions marker candidates. The clones contain only the specific V4V5 region marker candidates are labeled in red, only the specific V6 region marker candidates are in blue, and these have both specific marker regions are in orange. Bootstrap values between 0.7 to 1 are shown in the middle position of corresponding branches.

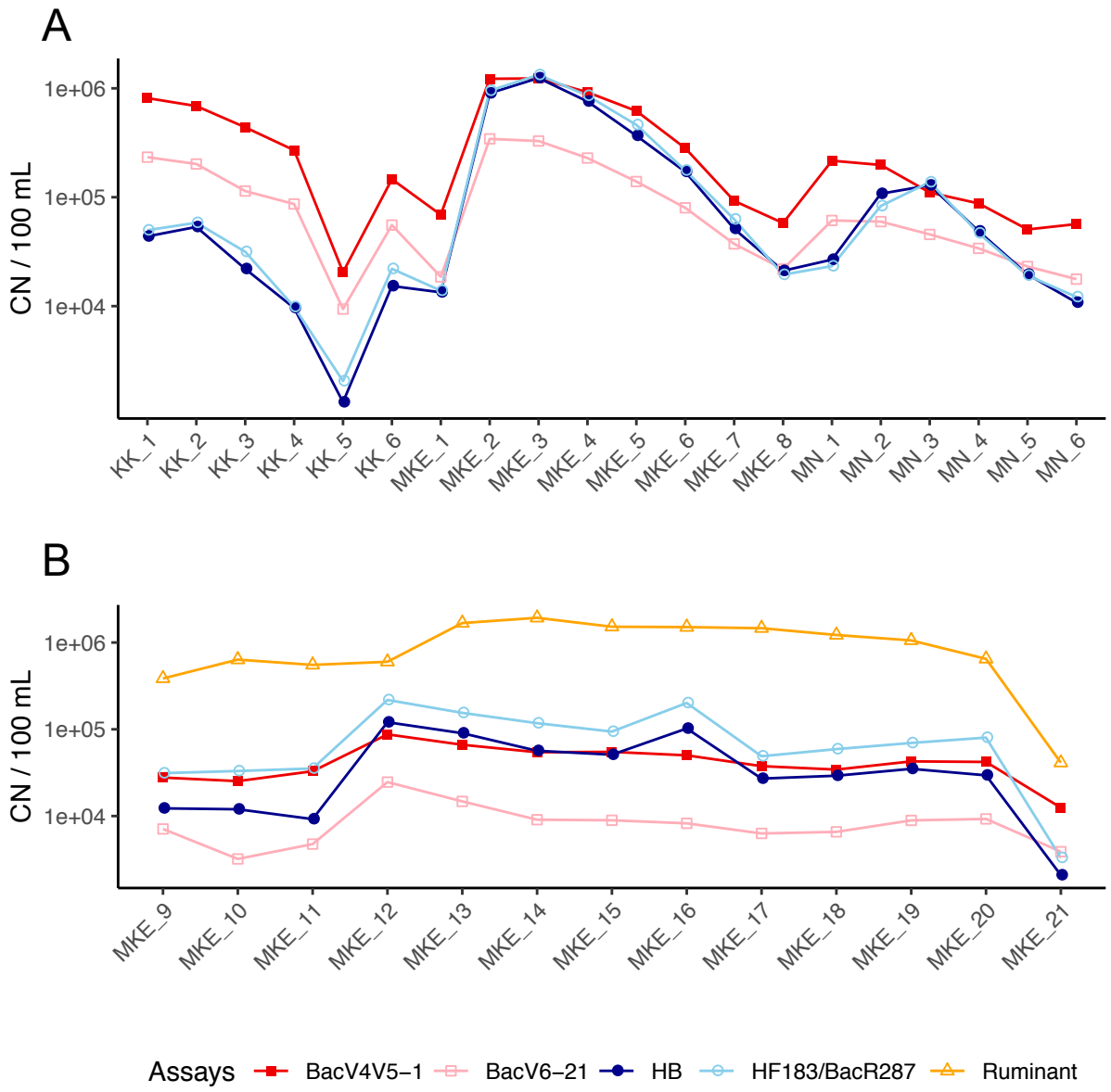

Fig. S6. Comparison of the four *Bacteroides* assays in environmental water samples. A shows comparison of the BacV4V5-1, BacV6-21, HB and HF183/BacR287 assays in sewage-contaminated water samples from Kinnickinnic River (KK), Milwaukee River (MKE) and Menomonee River (MN) from a 2016 combined sewer overflow (CSO) event. B shows comparison of the four *Bacteroides* assays and one ruminant marker assay in agricultural-contaminated MKE river water samples from rain and post-CSO events.

Table S1. Established *Bacteroides* marker assays, their reported animal cross-reactions and specificities. References in bold are the assay developers.

Target	Marker assays	Type	Tested animals	Positive animals	Specificity [a]	Reference
16S rRNA gene/*B. dorei*	HF183/Bac708R	PCR	Cow, cat, deer, dog, duck, elk, goat, llama, pig, seagull, sheep (n= 46)	None	100.0%	**Bernhard and Field 2000** (1)
		SYBR qPCR	Cow, pig, sheep, goat, horse, chicken, dog, duck, pelican, kangaroo (n=136)	Sheep	99.3%	Ahmed et al. 2009 (2)
		PCR	Pronghorn, moose, deer, duck, pelican, raccoon, gull, elk, cattle, goat, pig, turkey, sheep, chicken, dog, cat, dog and 3 marine animals (animals were tested as 22 pools of composite DNA for qPCR and as individuals for PCR) (n=158)	Dog	99.4%	Shanks et al. 2010 (3)
	HF183/SSHBac-R[b]	SYBR qPCR	Chicken, horse, cow, dog and pig (n=19)	Chicken	94.7%[c]	**Seurinck et al. 2005** (4)
		SYBR qPCR	Cow, horse, dog, cat and seagull (n=41)	Dog and cat	92.7%	Kildare et al. 2007 (5)
		SYBR qPCR	Cows, cat, dog and chicken (n=30)	Cat and dog	93.3%	Ahmed et al. 2010 (6)
		SYBR qPCR	Cat, dog, gull, rat and raccoon (n=47)	Cat	97.2%	Van De Werfhorst et al. 2011 (7)
		SYBR qPCR	Monkey, wild boar, bird, chicken, rabbit, cat and dog (n=220)	Chicken, rabbit and dog	88.6% 80.0% [d]	Nshimyimana et al. 2017 (8)
	HF183/BFDrev	TaqMan qPCR	Cow, pig, chicken, dog, cat (each animal as one group of composite DNA) (n=50)	Chicken and dog	60.0%	**Haugland et al. 2010** (9)
		TaqMan qPCR	Chicken, turkey, dog, cat, deer, pronghorn, pig and cow (n=123)	Chicken and turkey	93.5%	Green et al. 2014 (10)
	HF183/BacR287	TaqMan qPCR	As described above	Chicken and turkey	93.5%	**Green et al. 2014** (10)
		TaqMan qPCR	As described above	Chicken and rabbit	90.0% 86.7% [d]	Nshimyimana et al. 2017 (8)

		TaqMan qPCR	Cat, dog, pig, cow, deer and gull (n=55)	Deer	94.5%	Feng et al. 2018 (11)
	HB	TaqMan qPCR	As described above	Deer and dog	90.9%	**Templar et al. 2016 (12)** Feng et al. 2018 (11)
	HF183/BthetaF2	TaqMan qPCR	As described above	Chicken and dog	90.9%	Shanks et al. 2010 (3)
16S rRNA gene	BacHum-UCD	TaqMan qPCR	As described above	Dog	97.6%	**Kildare et al. 2007** (5)
		TaqMan qPCR	Dog, cow, horse and Canadian goose (as groups of composite DNA) (n=41)	Dog, cow and horse	70.7%	Silkie and Nelson 2009 (13)
		PCR	As described above	Pig, sheep, horse and dog	95.6%	Ahmed et al. 2009 (2)
		TaqMan qPCR	As described above	Cat, dog, gull and raccoon	38.9%	Van De Werfhorst et al. 2011 (7)
		TaqMan qPCR	As described above	Chicken, rabbit and dog	91.4% 73.3% [d]	Nshimyimana et al. 2017 (8)
16S rRNA gene	BacH	TaqMan qPCR	Cow, deer, chamois, roe deer, sheep, goat, horse, fox, dog, cat, pig, chicken, turkey, swan, duck and black grouse (n=302)	Cat	99.7%	**Reischer et al. 2007** (14)
		PCR	As described above	Sheep, goat and dog	94.1%	Ahmed et al. 2009 (2)
		TaqMan qPCR	As described above	Chicken and rabbit	90.0% 86.7% [d]	Nshimyimana et al. 2017 (8)
16S rRNA gene	HuBac	TaqMan qPCR	Cow, pig, horse and dog (n=18)	Cow, pig and dog	67.9%	**Layton et al. 2006** (15)
		TaqMan qPCR	As described above	Cow, horse, dog and cat	61.0%	Kildare et al. 2007 (5)
		PCR	As described above	Cow, pig, sheep, horse, dog and ducks	63.2%	Ahmed et al. 2009 (2)
		TaqMan qPCR	As described above	Deer, Canadian goose, duck, raccoon, elk, cow, pig, turkey, sheep, chicken, dog, cat and dog	22.7%	Shanks et al. 2010 (3)
16S rRNA	Human-Bac1	TaqMan qPCR	Cow and pig	Cow and pig	10.0% (10)	**Okabe et al. 2007** (16)

gene/ *B. fragilis*		PCR	As described above	Cow, sheep, horse, dog and kangaroo	78.7%	Ahmed et al. 2009 (2)
16S rRNA gene	BacHuman	TaqMan qPCR	Cow, pig, deer, horse, dog, cat, gull, goose and raccoon (n=54)	Pig, dog and cat	81.5% [c]	**Lee et al. 2010** (17)
Genomic sequence/ *B. thetaiotomicron*	B. theta	PCR	Dog, cow, chicken, turkey, horse, pig and goose (n=241)	Dog	97.9%	**Carson et al. 2005** (18)
		PCR	As described above	Dog	98.7%	Shanks et al. 2010 (3)
16S rRNA gene/ *B. thetaiotomicron*	BthetaF2	TaqMan qPCR	As described above	Pig, chicken, dog and cat	20.0%	**Haugland et al. 2010** (9)
		TaqMan qPCR	As described above	Pronghorn, moose, goose, duck, raccoon, gull, elk, dairy cow, pig, sheep, chicken, dog, cat, sea lion and elephant seal	31.8%	Shanks et al. 2010 (3)
Genomic sequence/ *B. thetaiotomicron α-mannanase*	B. theta α[b]	TaqMan qPCR	Dog, cow, horse, pig, chicken, turkey and goose (n=160)	None	100%	**Yampara-Iquise et al. 2008** (19)
		TaqMan qPCR	As described above	Cat	98.6% 93.3% [d]	Nshimyimana et al. 2017 (8)

a. Specificity is calculated as the percentage of negative animal fecal samples.

b. This marked assay was named by Harwood et al. 2014.

c. Animal false positives were reported in the reference publications.

d. The upper percentage represents specificity from individual animals, and the lower percentage represents the specificity from pooled animals.

Table S2. The V4V5 marker candidates, their specificity, sensitivity from the permutation test and the NGS dataset, and their probable source.

Marker name	Permutated sewage specificity	Permutated sewage sensitivity	NGS V4V5 dataset sewage specificity	NGS V4V5 dataset sewage sensitivity	Probable Source	Sequence
V4V5-1	1	1	1	1	Sewer pipe	ACGGAGGATCCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGTTGACATATAAGTCAGCTGTGAAAGTTTAC GGCTCAACCGTGAAATTGCAGTTGATACTGTATGTCTTGAGTGTACAAGAGGTGGGCGGAATTCGTGGTGTAGCGGTGAAA TGCTTAGATATCACGAAGAACTCCAATTGCGAAGGCAGCTCACTGGGGTACAACTGACACTGAGGCTCGAAAGTGTGGGTA TCAAACAGGATTAGATACCCTGGTAGTCCACACAGTAAACGATGAATACTCGCTGTTTGCGATATACAGTAAGCGGCCAAG CGAAAGCATTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGAA
V4V5-7	1	1	1	1	Sewer pipe	ACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGTTGACGTATAAGTCAGCTGTGAAAGTTTAC GGCTCAACCGTGAAATTGCAGTTGATACTGTATGTCTTGAGTGTACAAGAGGTGGGCGGAATTCGTGGTGTAGCGGTGAAA TGCTTAGATATCACGAAGAACTCCAATTGCGAAGGCAGCTCACTGGGGTACAACTGACACTGAGGCTCGAAAGTGTGGGTA TCAAACAGGATTAGATACCCTGGTAGTCCACACAGTAAACGATGAATACTCGCTGTTTGCGATATACAGTAAGCGGCCAAG CGAAAGCATTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGAA
V4V5-13	1	0.96	1	0.96	Sewer pipe	ACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGTTGACATATAAGTCAGCTGTGAAAGTTTAC GGCTCAACCGTGAAATTGCAGTTGATACTGTATGTCTTGAGTGTACAAGAGGTGGGCGGAATTCGTGGTGTAGCGGTGAAA TGCTTAGATATCACGAAGAACTCCAATTGCGAAGGCAGCTCACTGGGGTACAACTGACACTGAGGCTCGAAAGTGTGGGTA TCAAACAGGATTAGATACCCTGGTAGTCCACACAGTAAACGATGAATACTCGCTGTTTGCGATATACAGTAAGCGGCCAAG CGAAAGCATTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGAA
V4V5-22	1	0.95	1	0.95	Human feces	ACGGAGGATGCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGCAGACGGGTCGTTAAGTCAGCTGTGAAAGTTTG GGGCTCAACCTTAAAATTGCAGTTGATACTGGCGTCCTTGAGTGCGGTTGAGGTGTGCGGAATTCGTGGTGTAGCGGTGA AATGCTTAGATATCACGAAGAACTCCGATTGCGAAGGCAGCACACTAATCCGTAACTGACGTTCATGCTCGAAAGTGTGGG TATCAAACAGGATTAGATACCCTGGTAGTCCACACGGTAAACGATGGATACTCGCTGTTGGCGATATACTGTCAGCGGCTT AGCGAAAGCGTTAAGTATCCCACCTGGGGAGTACGCCGGCAACGGTGAA
V4V5-25	1	0.91	1	0.91	Sewer pipe	ACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGCGGATGTTTAAGTCAGTTGTGAAAGTTTAA GGCTCAACCTTGAAATTGCAGTTGATACTGGATATCTTGAGTACATTGAATGTGGGCGGAATTCGTGGTGTAGCGGTGAAA TGCTTAGATATCACGAAGAACTCCAATTGCGAAGGCAGCTCACAGTAATGTAACTGACGCTGATGCTCGAAAGTGTGGGTA TCAAACAGGATTAGATACCCTGGTAGTCCACACAGTAAACGATGAATACTCGCTGTTTGCGATATACAGTAAGCGGCCAAG CGAAAGCGTTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGAA
V4V5-32	1	0.94	1	0.94	Human feces	ACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGCGGGTGCTTAAGTCAGTTGTGAAAGTTTGC GGCTCAACCGTAAAATTGCAGTTGATACTGGGTACCTTGAGTGCAGCATAGGTAGGCGGAATTCGTGGTGTAGCGGTGAAA TGCTTAGATATCACGAAGAACTCCGATTGCGAAGGCAGCTTACTGGACTGTAACTGACGCTGATGCTCGAAAGTGTGGGTA TCAAACAGGATTAGATACCCTGGTAGTCCACACAGTAAACGATGAATACTCGCTGTTGGCGATACACAGTCAGCGGCCAAG CGAAAGCATTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGAA
V4V5-37	1	0.93	1	0.93	Human feces	ACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGCGGATTATTAAGTCAGTTGTGAAAGTTTGC GGCTCAACCGTAAAATTGCAGTTGATACTGGTAGTCTTGAGTGCAGCAGAGGTAGGCGGAATTCGTGGTGTAGCGGTGAA ATGCTTAGATATCACGAAGAACTCCGATTGCGAAGGCAGCTTACTGGACTGTAACTGACGCTGATGCTCGAAAGTGTGGGT ATCAAACAGGATTAGATACCCTGGTAGTCCACACAGTAAACGATGAATACTCGCTGTTTGCGATATACAGCAAGCGGCCAA GCGAAAGCATTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGAA

Table S3. The V6 marker candidates, their specificity, sensitivity from the permutation test and the NGS dataset, and their probable source.

Marker name	Permutated sewage specificity	Permutated sewage sensitivity	NGS V6 dataset sewage specificity	NGS V6 dataset sewage sensitivity	Probable Source	Sequence
V6-21	1	1	1	1	Sewer pipe	CGGGCTTGAATTGCAGAGGAATATAGTTGAAAGATTATGGCCGCAAGGTCTCTGTGA
V6-23	1	1	1	1	Human feces	CGGGCTTAAATTGCAAATGAATTATGGGGAAACCCATAGGCCGTAAGGCATTTGTGA
V6-24	1	1	1	1	Sewer pipe	CAGGCTTAAATTGCAGATGAATATAGTGGAAACATTATAGCCTTCGGGCATCTGTGA
V6-26	1	1	1	1	Sewer pipe	CGGGCTTAAATTGCACAGGAATAATTTGGAAACAGATTAGTCTTCGGACCTGTGTGA
V6-36	1	1	1	1	Sewer pipe	CGGGCTTGAATTGCTAATGAATATATATGAAAGTATATAGCCGCAAGGCATTAGTGA
V6-38	1	1	1	1	Sewer pipe	CGGGCTTGAATTGCTAATGAATGGAGTAGAGATATTTCAGCCGCAAGGCATTAGTGA
V6-44	1	1	1	1	Sewer pipe	CAGGCTTAAATTGCAGATGAATATAGTAGAAATATTATAGCCTTCGGGCATCTGTGA
V6-17	1	0.95	1	1	Sewer pipe	CGGGCTTAAATTGCAAATGAATATAGTGGAAACATTATAGCCAGCAATGGCATTTGTGA
V6-32	1	0.95	1	1	Sewer pipe	CAGGCTTAAATTGCAGATGAATATAGTGGAAACATTATAGTCTTCGGACATCTGTGA
V6-34	1	0.95	1	1	Sewer pipe	CGGGCTTAAATTGCAACTGAATAGCTGAGAGATCAGTTAGCTAGCAATAGCAGTTGTGA
V6-37	1	0.95	1	1	Sewer pipe	CGGGCTTGAATTGCAGAGGAATATAGTTGAAAGATTATAGCCGCAAGGCCTCTGTGA
V6-40	1	0.925	1	1	Sewer pipe	CAGGCTTAAATTGCAGATGAATATGTGGGAAACCATATAGCCAGCAATGGCATCTGTGA
V6-42	1	0.95	1	1	Sewer pipe	CAGGCTTAAATTGCAGATGAATATAGTGGAAACATTATAGCCAGCAATGGCATCTGTGA
V6-45	1	0.9	1	1	Sewer pipe	CAGGCTTAAATTGCAGATGAATATAGTAGAAATATTATAGTCTTCGGACATCTGTGA
V6-50	1	0.975	1	1	Sewer pipe	CGGGCTTGAATTGCAGAGGAATATAGTCGAAAGATTATAGCCGCAAGGTCTCTGTGA
V6-52	1	0.925	1	0.875	Human feces	CGGGCTTAAATTGCAAATGAATATGCCGGAAACGGCATAGCCGCAAGGCATTTGTGA
V6-55	1	0.95	1	0.95	Sewer pipe	CAGGCTTAAATTGCAGATGAATATAGTGGAAACATTATAGCCTTTATGGCATCTGTGA
V6-68	1	0.9	1	1	Sewer pipe	CGGGCTTGAATTGCAGAGGAACATAGTTGAAAGATTATCGCCGCAAGGTCTCTGTGA
V6-73	1	0.925	1	1	Sewer pipe	CGGGCTTAAATTGCAACTGAATAATTGAGAGATCAGTTAGCTAGCAATAGCAGTTGTGA
V6-79	1	0.925	1	1	Sewer pipe	CGGGCTTAAATTGCAACTGAATAACTTAGAGATGAGTTAGCTAGCAATAGCAGTTGTGA
V6-96	1	0.9	1	1	Human feces	CGGGTTTGAACGCATTCGGACCGGAGTGGAAACACTTCTTCTAGCAATAGCCGTTTGCG

Table S4. Amplicon sequences of the BacV4V5-1 and BacV6-21 assays.

Assay name	Amplicon sequence	Examples of reference clone sequences (GenBank Access. No.)
BacV4V5-1	AAGGGAGCGTAGGTTGACATATAAGTCAGCTGTGAAAGTTTACGGCTCAACC GTGAAATTGCAGTTGATACTGTATGTCTTGAGTGTACAAGAGGTGGGCGG	MH515903, MH515911, MH515713
BacV6-21	GCTTGAATTGCAGAGGAATATAGTTGAAAGATTATGGCCGCAAGGTCTCTGT GAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCTTAAG TGCCATAACGAGCGCAACCCTTATCATTAGTTACTAACAGGTCATGCTGAGG ACTCTAGTGAGACTGC	MH515733, MH515713

Table S5. Slopes, y intercepts, R^2 and efficiencies of the four qPCR assays used in this study.

Assay name	Slope	Y intercept	R^2	Efficiency (%)
BacV4V5-1	-3.364	38.056	0.998	98.3235
BacV6-21	-3.399	38.934	0.997	96.869
HB	-3.372	37.468	0.999	98.026
HF183/BacR287	-3.514	38.565	0.999	92.591

Reference

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