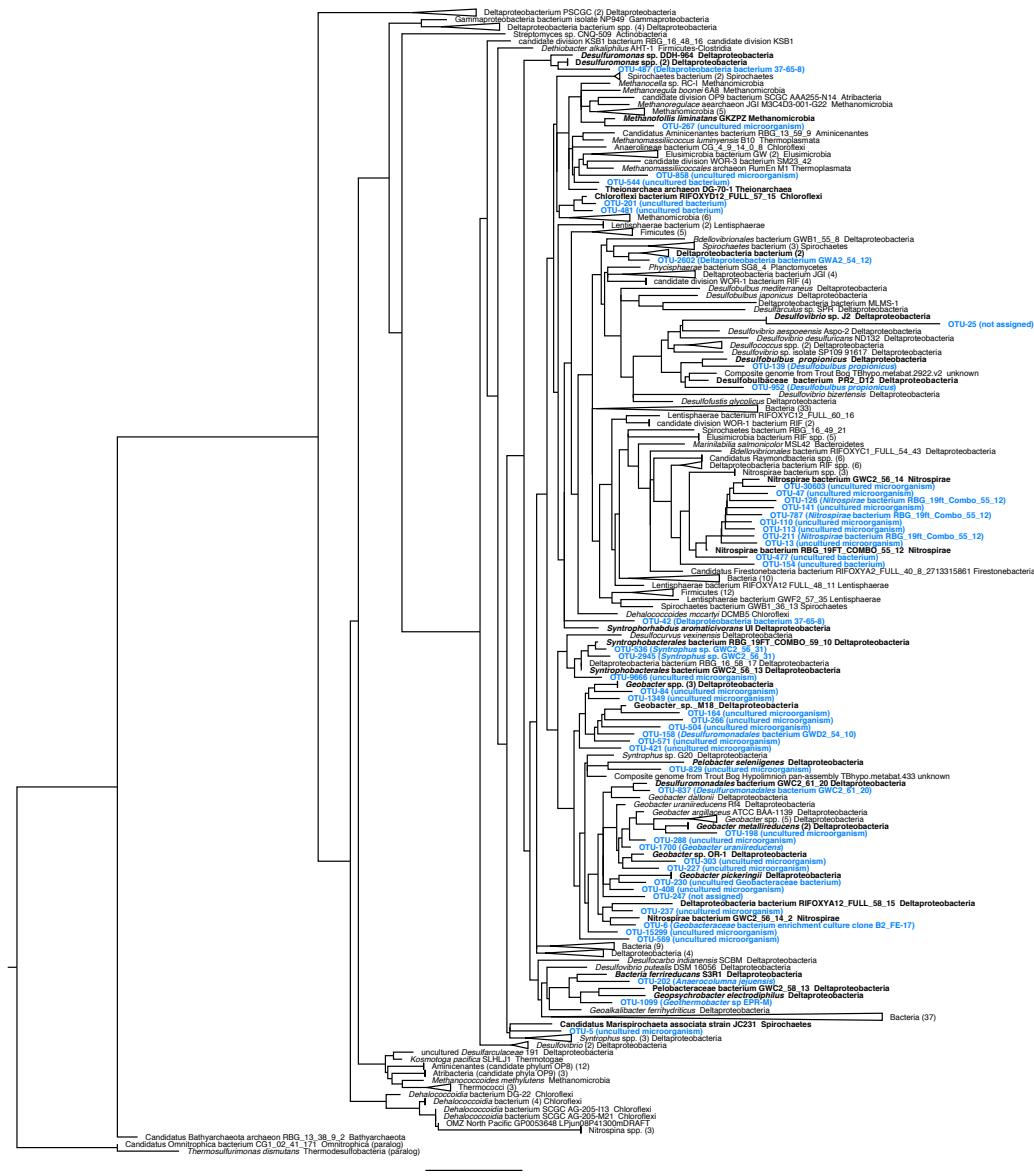


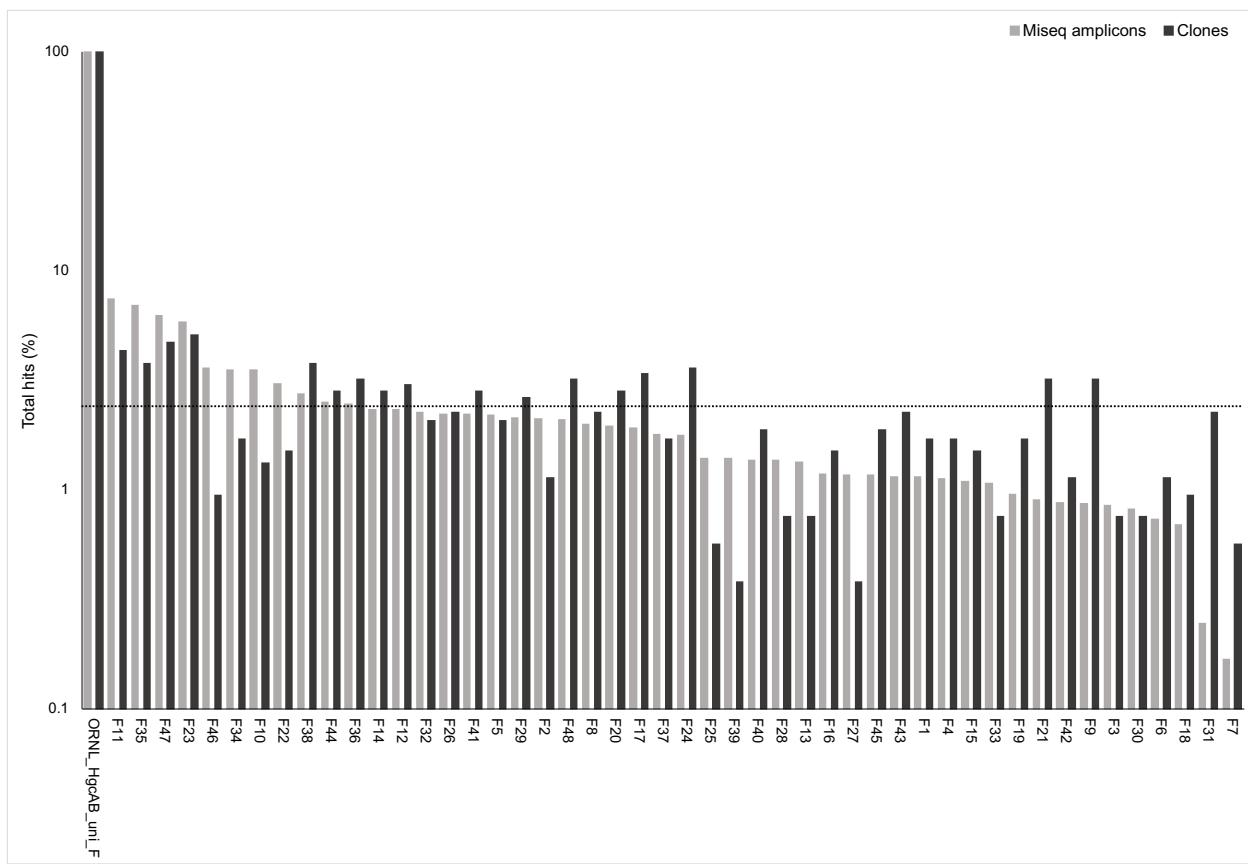
Supplementary Material

1 Supplementary Figures and Tables

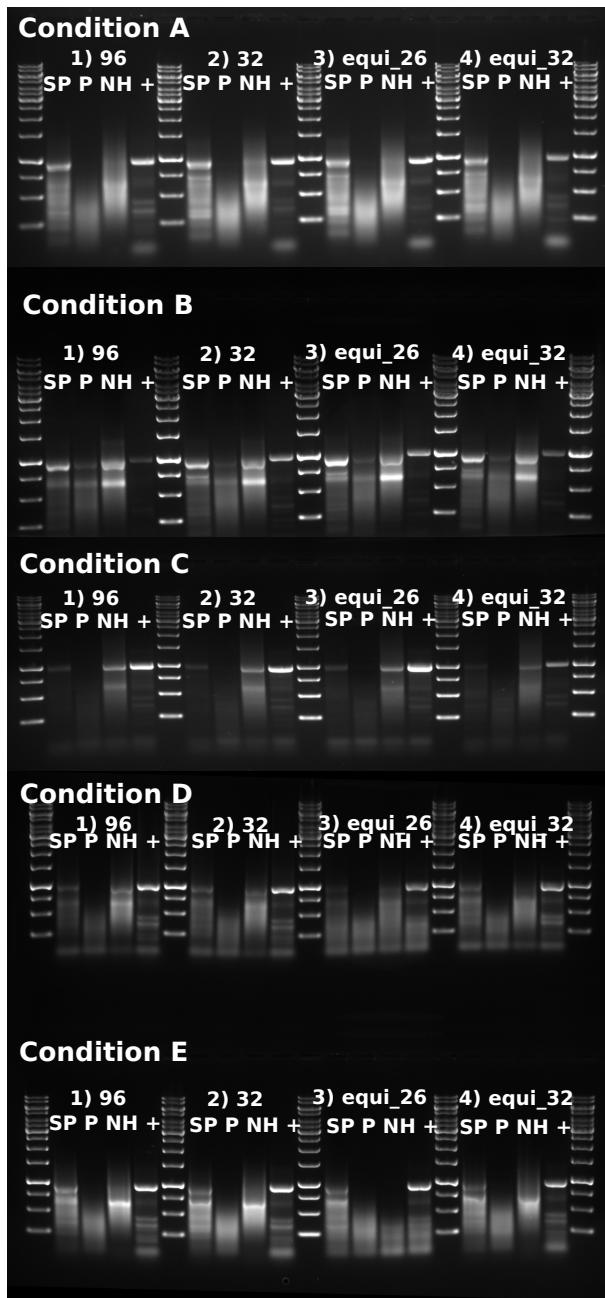
1.1 Supplementary Figures



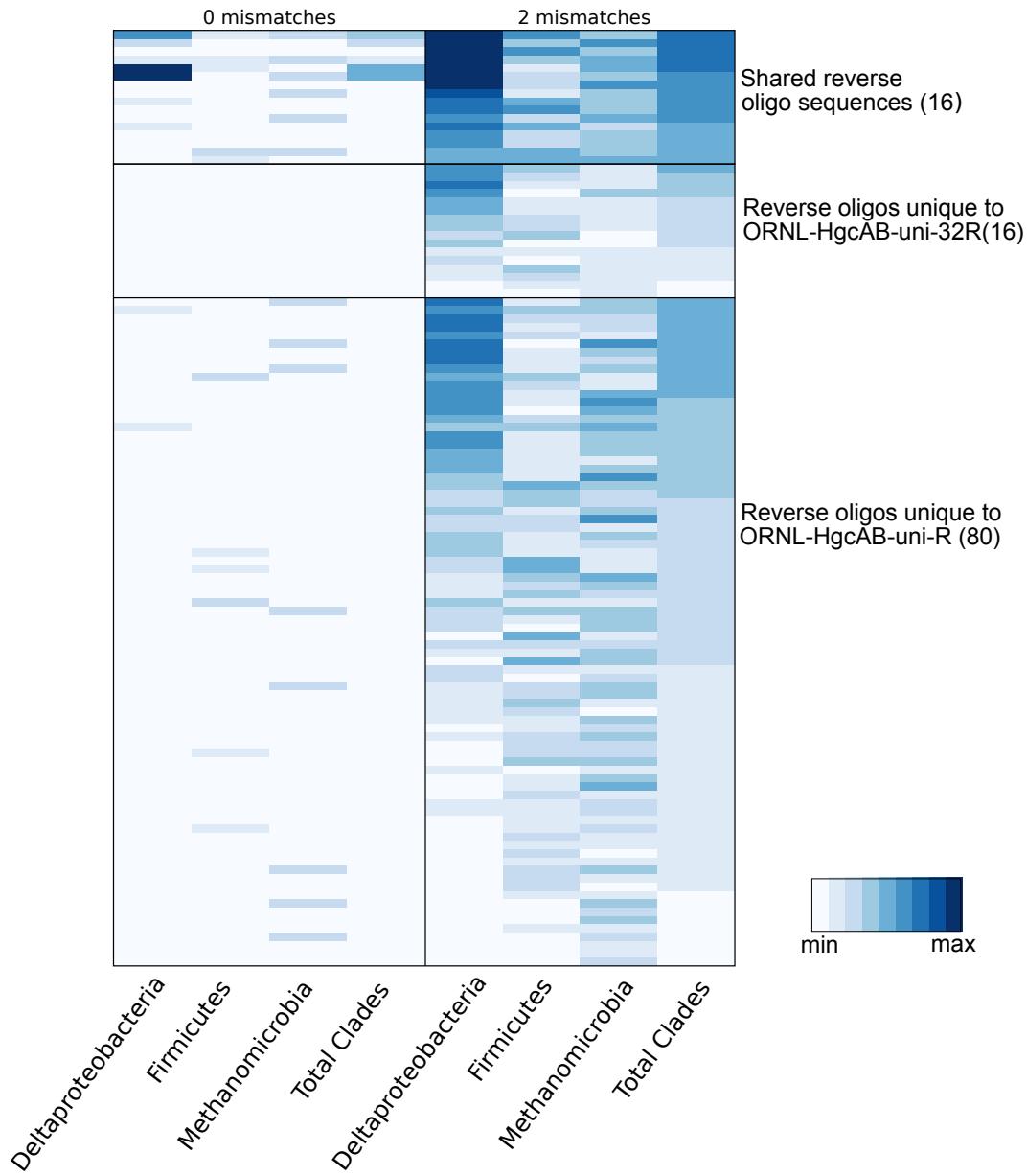
Supplementary Figure 1. Maximum likelihood tree of *hgcA* sequences from the updated reference package showing pplacer placements of the top 50 OTUS from New Horizon (NH) *hgcA* amplicon sequences (Matsen et al., 2010). Nearest neighbor shown in bold. NH *hgcA* sequences are shown in blue with classification based on the lowest common ancestor (LCA, 50 % cut-off) of protein BLAST hits to NCBI non-redundant protein database (Altschul et al., 1990); LCA calculated in MEGAN v6 (Huson et al., 2016).



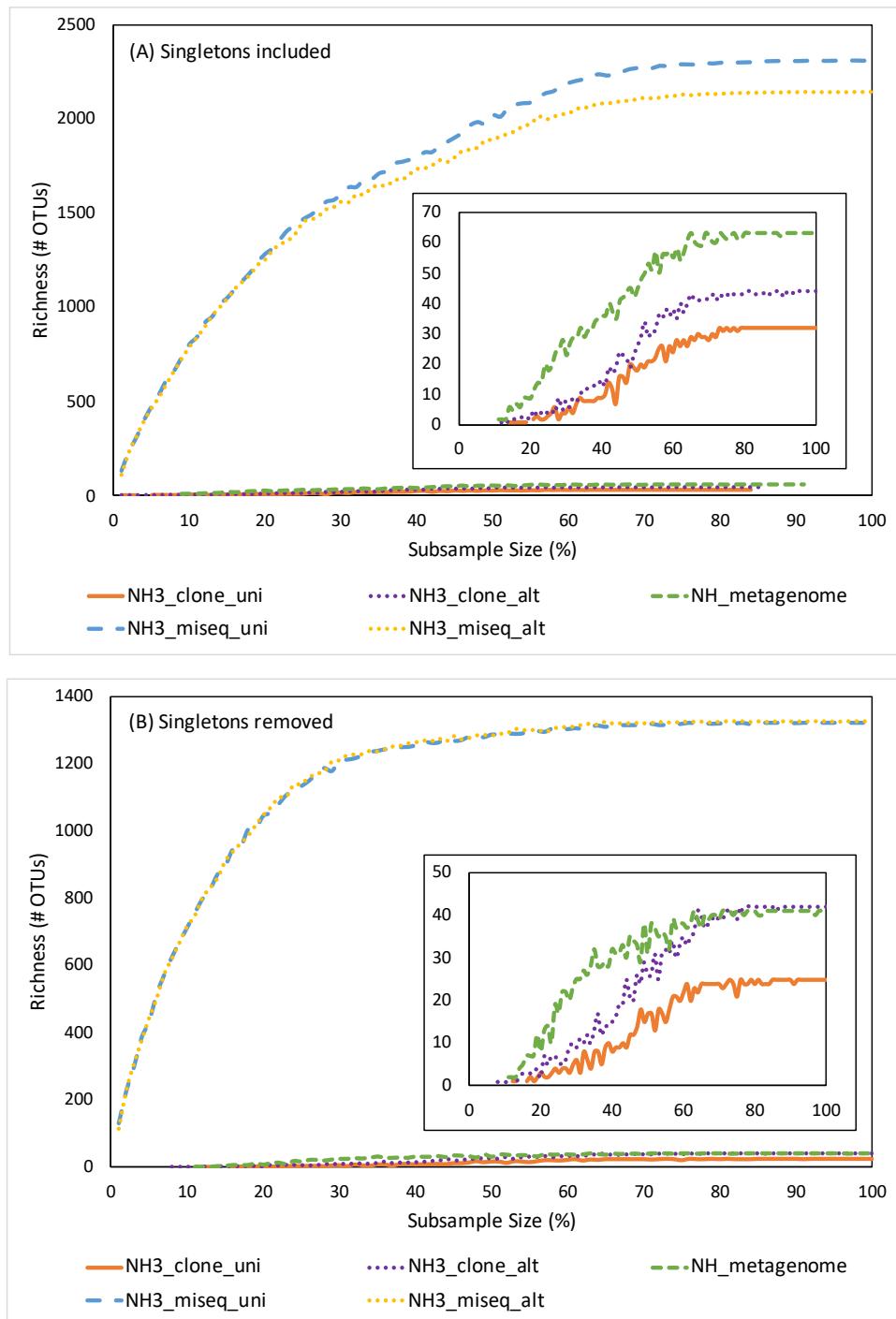
Supplementary Figure 2. Percent occurrence of each of the 48-degenerate forward (ORNL-HgcAB-uni-F) oligonucleotide sequence in the environmental clone *hgcAB* sequences (Table S2) and Miseq *hgcAB* amplicons from sites NH. Clones were generated with both the Christensen 2016 primer set (ORNL-HgcAB-uni-F, ORNL-HgcAB-uni-R; n = 119) and the new less degenerate primer set (ORNL-HgcAB-uni-F, ORNL-HgcAB-uni-32R; n = 509). Forward primer binding sites were found in 531 clones of the total 628 clone library. A subset of sequences were pulled from amplicon libraries produced from six separate NH sediment samples using ORNL-HgcAB-uni-F and ORNL-HgcAB-uni-R (n = 87,500) and ORNL-HgcAB-uni-F and ORNL-HgcAB-uni-32R (n = 87,500). Forward primer binding sites were found in 166,984 of the 175,000 amplicon sequences searched. Primers are listed in Table S2. Data is shown on a log scale. Dashed line indicates equal distribution of forward primer sequences across amplicon sequences: 2.08%.



Supplementary Figure 3. Gel image showing PCR amplification of *hgcAB* from SPRUCE soil (SP), NH periphyton (P), and NH sediment (NH) using PCR conditions A—E (Table S4) with 96 degenerate primer set (ORNL-HgcAB-uni-F, ORNL-HgcAB-uni-R), 32 degenerate primer set (ORNL-HgcAB-uni-F, ORNL-HgcAB-uni-R32), and equimolar reverse primer mixes of ORNL-HgcAB-uni-R26 and ORNL-HgcAB-uni-R32. *Desulfovibrio desulfuricans* ND132 *hgcAB* amplicon (~980bp) was included as a positive (+) control, compared against a 1 kbp GeneRuler ladder.



Supplementary Figure 4. Heatmap showing the percentage that each possible sequence from the reverse broad-range primer from this study (ORNL-HgcAB-uni-32R) compared to the more degenerate primer from previous study (ORNL-HgcAB-uni-R; Christensen et al. 2016) align with *hgcAB* from reference sequences (listed in Table S1) of the three major clades: *Deltaproteobacteria* (124), *Firmicutes* (32), *Methanomicrobia* (15), and total sequences (239). Alignments allowed for either no mismatches (on the left), and up to 2 mismatches (on the right). The colorbar is scaled based on the minimum and maximum values for the 0-mismatch and 2-mismatch sets separately (0–19% and 1–85%, respectively).



Supplementary Figure 5. Rarefaction curves assessing OTU richness in *hgcA* sequences from NH sediment (East Fork Poplar Creek, Oak Ridge, TN) clone, amplicon, and metagenomic datasets. The clone and amplicon sequences were amplified from sample NH3 with the reverse primer from this study ('uni-32R') compared to the more degenerate primer from previous study ('uni-R';(Christensen et al., 2016)). Included are *hgcA* sequences pulled from a NH sediment metagenomic dataset from a previous study (Christensen et al., 2019). OTU richness was calculated using USEARCH with singletons included (A) and discarded (B) prior to OTU clustering (Edgar, 2010).

1.2 Supplementary Tables

Supplementary Table 1. List of *hgcAB*+ organisms used in reference package and for *in silico* analyses. Sequences were selected from publicly available genomes from the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>), including Hg-methylating organisms, shotgun metagenomics, and metagenome-assemble microbial genomes (MAGS). This list, including *hgcA* and *hgcB* nucleotide sequences, is available online through the DOE Data Explorer (Gionfriddo et al., 2019).

* indicates only an *hgcA* sequence is available for this organism.

Acetivibrio cellulolyticus CD2 DSM 1870 (*Firmicutes-Clostridia*)
Acetonema longum DSM 6540 (*Firmicutes-Clostridia*)
Alkaliphilus peptidifermans DSM 18978 (*Firmicutes-Clostridia*)
Anaerolineae bacterium CG_4_9_14_0_8 (*Chloroflexi*)
Bacteria ferrireducans S3R1 (*Delta proteobacteria*)
Bacteroides cellulosolvens DSM 2933 (*Bacteroidetes*)
Bacteroides sp. SM1 62 (*Bacteroidetes*)
Bacteroides sp. SM23 62 (*Bacteroidetes*)
Bacteroidetes bacterium GWA2_32_17 (*Bacteroidetes*)
Bacteroidetes bacterium GWF2_35_48 (*Bacteroidetes*)
Bacteroidetes bacterium RIFOXYA12_FULL_35_11 (*Bacteroidetes*)
Bacteroidetes bacterium RIFOXYC12_FULL_35_7 (*Bacteroidetes*)
Bdellovibrionales bacterium GWB1_55_8 (*Delta proteobacteria*)
Bdellovibrionales bacterium RIFOXYB1_FULL_39_21 (*Delta proteobacteria*)*
Bdellovibrionales bacterium RIFOXYC1_FULL_39_130 (*Delta proteobacteria*)*
Bdellovibrionales bacterium RIFOXYC1_FULL_54_43 (*Delta proteobacteria*)
Bdellovibrionales bacterium RIFOXYC12_FULL_39_17 (*Delta proteobacteria*)*
Bdellovibrionales bacterium RIFOXYD12_FULL_39_22 (*Delta proteobacteria*)*
candidate division KSB1 bacterium RBG_16_48_16 (candidate division KSB1)
candidate division OP8 bacterium SCGC_AAA252-A02 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-F08 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-G05 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-G06 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-J09 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-J21 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-K07 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-O09 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-O19 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-P13 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA252-P19 (*Aminicenantes*)*
candidate division OP8 bacterium SCGC_AAA255-O15 (*Aminicenantes*)

candidate division OP9 bacterium SCGC AAA252-M02 (*Atribacteria*)*
candidate division OP9 bacterium SCGC AAA255-N14 (*Atribacteria*)
candidate division OP9 bacterium SCGC AB-164-L03 (*Atribacteria*)*
candidate division OP9 bacterium SCGC AB-164-P05 (*Atribacteria*)*
candidate division WOR-1 bacterium RIFOXYA2_FULL_41_14 (candidate division WOR-1)
candidate division WOR-1 bacterium RIFOXYA2_FULL_46_56 (candidate division WOR-1)
candidate division WOR-1 bacterium RIFOXYA12_FULL_43_27 (candidate division WOR-1)
candidate division WOR-1 bacterium RIFOXYB2_FULL_42_35 (candidate division WOR-1)
candidate division WOR-1 bacterium RIFOXYB2_FULL_46_45 (candidate division WOR-1)
candidate division WOR-1 bacterium RIFOXYC2_FULL_46_14 (candidate division WOR-1)
candidate division WOR-3 bacterium SM23_42 (candidate division WOR-3)
Candidatus *Aminicenantes* bacterium RBG_13_59_9 (*Aminicenantes*)
Candidatus *Bathyarchaeota* archaeon RBG_13_38_9_2 (*Bathyarchaeota*)*
Candidatus *Desulfuromonas soudanensis* WTL (*Deltaproteobacteria*)
Candidatus *Firestonebacteria* bacterium RIFOXYA2_FULL_40_8 (*Firestonebacteria*)
Candidatus *Marispirochaeta associata* strain JC231 (*Spirochaetes*)
Candidatus *Methanoregula boonei* 6A8 (*Methanomicrobia*)
Candidatus *Methanospaerula palustris* E1-9c (*Methanomicrobia*)
Candidatus *Raymondbacteria* bacterium RIFOXYA12_full_50_37 (*Raymondbacteria*)
Candidatus *Raymondbacteria* bacterium RIFOXYA2_FULL_49_16 (*Raymondbacteria*)
Candidatus *Raymondbacteria* bacterium RIFOXYB12_full_50_8 (*Raymondbacteria*)
Candidatus *Raymondbacteria* bacterium RIFOXYB2_FULL_49_35 (*Raymondbacteria*)
Candidatus *Raymondbacteria* bacterium RifOxyC12_full_50_8 (*Raymondbacteria*)
Candidatus *Raymondbacteria* bacterium RIFOXYD12_FULL_49_13 (*Raymondbacteria*)
Candidatus *Wallbacteria* bacterium GWC2_49_35 (*Wallbacteria*)*
Chloroflexi bacterium RIFOXYD12_FULL_57_15 (*Chloroflexi*)
Clostridium cellobioparum ATCC 15832 (*Firmicutes-Clostridia*)
Clostridium cellulosi CS-4-4 (*Firmicutes-Clostridia*)*
clostridium Ga0073690 (*Firmicutes-Clostridia*)
Clostridium jejuense DSM 15929 (*Firmicutes-Clostridia*)
Clostridium litorale W6 DSM 5388 (*Firmicutes-Clostridia*)
Clostridium sp. 3 Draft 3 (*Firmicutes-Clostridia*)
Clostridium termitidis CT1112 DSM 5398 (*Firmicutes-Clostridia*)
Clostridium tunisiense TJ (*Firmicutes-Clostridia*)
Clostridium xylanovorans DSM 12503 (*Firmicutes-Clostridia*)
Composite genome from Trout Bog Hypolimnion pan-assembly TBhypo.metabat.433 (unknown)
Composite genome from Trout Bog Hypolimnion TBhypo.metabat.3815 (unknown)
Composite genome from Trout Bog TBhypo.metabat.2922.v2 (unknown)
Composite genome from Trout Bog TBhypo.metabat.5247 (unknown)
Dehalobacter restrictus DSM 9455 (*Firmicutes-Clostridia*)

Dehalobacter sp. 11DCA (*Firmicutes-Clostridia*)
Dehalobacter sp. CF (*Firmicutes-Clostridia*)
Dehalobacter sp. UNSWDHB (*Firmicutes-Clostridia*)
Dehalococcoides mccartyi DCMB5 (*Chloroflexi*)
Dehalococcoidia bacterium DG 22 (*Chloroflexi*)*
Dehalococcoidia bacterium SCGC_AG-205-B13 (*Chloroflexi*)*
Dehalococcoidia bacterium SCGC_AG-205-I02 (*Chloroflexi*)*
Dehalococcoidia bacterium SCGC_AG-205-I13 (*Chloroflexi*)*
Dehalococcoidia bacterium SCGC_AG-205-K13 (*Chloroflexi*)*
Dehalococcoidia bacterium SCGC_AG-205-M10 (*Chloroflexi*)*
Dehalococcoidia bacterium SCGC_AG-205-M21 (*Chloroflexi*)*
delta proteobacterium MLMS-1 (*Deltaproteobacteria*)
Delta proteobacterium NaphS2 (*Deltaproteobacteria*)
delta proteobacterium PSCGC 5419 (*Deltaproteobacteria*)
delta proteobacterium PSCGC 5451 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium GWA2_55_10 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium isolate ARS66 (*Deltaproteobacteria*)*
Deltaproteobacteria bacterium isolate NP36 76525 (*Deltaproteobacteria*)*
Deltaproteobacteria bacterium isolate NP36 (*Deltaproteobacteria*)*
Deltaproteobacteria bacterium isolate SP3084 (*Deltaproteobacteria*)*
Deltaproteobacteria bacterium JGI A06048-F13 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium JGI E06040-H20 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RBG_16_44_11 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RBG_16_58_17 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RBG_19FT_COMBO_43_11 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFCSPHIGO2_02_FULL_42_44 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFCSPHIGO2_02_FULL_43_33 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFCSPLOWO2_01_FULL_42_9 (*Deltaproteobacteria*)*
Deltaproteobacteria bacterium RIFCSPLOWO2_02_FULL_42_39 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFCSPLOWO2_02_FULL_55_12 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFCSPLOWO2_12_FULL_43_16 (*Deltaproteobacteria*)*
Deltaproteobacteria bacterium RIFOXYA2_FULL_42_10 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFOXYA12_FULL_58_15 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFOXYD12_FULL_50_9 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFOXYD12_FULL_53_23 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFOXYD12_FULL_56_24 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium RIFOXYD12_FULL_57_12 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium SG8 13 (*Deltaproteobacteria*)
Deltaproteobacteria bacterium SM23 61 (*Deltaproteobacteria*)
Deltaproteobacterium sp. OalgD1a (*Deltaproteobacteria*)

Deltaproteobacterium sp. OalgD1b (*Deltaproteobacteria*)
Deltaproteobacterium sp. OalgD3 (*Deltaproteobacteria*)
Deltaproteobacterium sp. OalgD4 (*Deltaproteobacteria*)
Desulfacinum hydrothermale DSM 13146 (*Deltaproteobacteria*)
Desulfacinum infernum DSM 9756 (*Deltaproteobacteria*)
Desulfarculus sp. SPR (*Deltaproteobacteria*)
Desulfitobacterium dehalogenans JWIU-DC1 ATTC 51507 (*Firmicutes-Clostridia*)
Desulfitobacterium dichloroeliminans LMG P21439 (*Firmicutes-Clostridia*)
Desulfitobacterium metallireducens 853-15A DSM 15288 (*Firmicutes-Clostridia*)
Desulfitobacterium sp. PCE1 DSM 10344 (*Firmicutes-Clostridia*)
Desulfobacter sp. isolate ARS36 (*Deltaproteobacteria*)
Desulfobacterales bacterium RIFOXYA12_FULL_46_15 (*Deltaproteobacteria*)
Desulfobacterales bacterium SG8_35_2 (*Deltaproteobacteria*)
Desulfobacterium vacuolatum DSM 3385 (*Firmicutes-Clostridia*)
Desulfobacula phenolica DSM 3384 (*Deltaproteobacteria*)
Desulfobacula sp. TS (*Deltaproteobacteria*)
Desulfobulbaceae bacterium PR2 D12 (*Deltaproteobacteria*)
Desulfobulbus japonicus DSM 18378 (*Deltaproteobacteria*)
Desulfobulbus mediterraneus DSM 13871 (*Deltaproteobacteria*)
Desulfobulbus propionicus DSM 2032 (*Deltaproteobacteria*)
Desulfobulbus sp. Tol-SR (*Deltaproteobacteria*)
Desulfocarbo indianensis SCBM (*Deltaproteobacteria*)
Desulfococcus biacutus KMRActS (*Deltaproteobacteria*)
Desulfococcus multivorans DSM 2059 (*Deltaproteobacteria*)
Desulfocurvus vexinensis DSM 17965 (*Deltaproteobacteria*)
Desulfofustis glycolicus DSM 9705 (*Deltaproteobacteria*)
Desulfoluna spongiiphila AA1 (*Deltaproteobacteria*)
Desulfomicrobium apsheronum DSM 5918 (*Deltaproteobacteria*)
Desulfomicrobium baculum DSM 4028 (*Deltaproteobacteria*)
Desulfomicrobium escambienense DSM 10707 (*Deltaproteobacteria*)
Desulfomicrobium norvegicum DSM 1741 (*Deltaproteobacteria*)
Desulfomonile sp. Ga0081660 (*Deltaproteobacteria*)*
Desulfomonile tiedjei DCB-1 DSM 6799 (*Deltaproteobacteria*)
Desulfonatronospira thiodismutans ASO3-1 (*Deltaproteobacteria*)
Desulfonatronovibrio hydrogenovorans DSM 9292 (*Deltaproteobacteria*)
Desulfonatronum lacustre Z-7951 DSM 10312 (*Deltaproteobacteria*)
Desulfonatronum thioautotrophicum ASO4-1 (*Deltaproteobacteria*)
Desulfonatronum thiodismutans MLF-1 (*Deltaproteobacteria*)
Desulfonatronum thiosulfatophilum ASO4-2 (*Deltaproteobacteria*)
Desulfonatronum zhilinae Al915-01 (*Deltaproteobacteria*)

Desulfopila aestuarii DSM 18488 (*Deltaproteobacteria*)
Desulfosarcina cetonica JCM 12296 (*Deltaproteobacteria*)
Desulfospira joergensenii DSM 10085 (*Deltaproteobacteria*)
Desulfosporosinus acididurans M1 (*Firmicutes-Clostridia*)
Desulfosporosinus acidophilus SJ4 DSM 22704 (*Firmicutes-Clostridia*)
Desulfosporosinus lacus DSM 15449 (*Firmicutes-Clostridia*)
Desulfosporosinus orientis Singapore I DSM 765 (*Firmicutes-Clostridia*)
Desulfosporosinus sp. I2 (*Firmicutes-Clostridia*)
Desulfosporosinus sp. OT (*Firmicutes-Clostridia*)
Desulfosporosinus sp. Tol-M Ga0063340 (*Firmicutes-Clostridia*)
Desulfosporosinus youngiae JWYJL-B18 DSM 17734 (*Firmicutes-Clostridia*)
Desulfotignum balticum DSM 7044 (*Deltaproteobacteria*)
Desulfotignum phosphitoxidans FiPS-3 (*Deltaproteobacteria*)
Desulfovibrio aespoeensis Aspo-2 chromosome (*Deltaproteobacteria*)
Desulfovibrio africanus DSM 2603 2527068623 (*Deltaproteobacteria*)
Desulfovibrio africanus PCS 2520045431 (*Deltaproteobacteria*)
Desulfovibrio africanus Walvis Bay (*Deltaproteobacteria*)
Desulfovibrio alkalitolerans DSM 16529 (*Deltaproteobacteria*)
Desulfovibrio bizertensis DSM 18034 (*Deltaproteobacteria*)
Desulfovibrio desulfuricans ND132 (*Deltaproteobacteria*)
Desulfovibrio halophilus DSM 5663 (*Deltaproteobacteria*)
Desulfovibrio inopinatus DSM 10711 (*Deltaproteobacteria*)
Desulfovibrio longus DSM 6739 (*Deltaproteobacteria*)
Desulfovibrio oxyclinae DSM 11498 (*Deltaproteobacteria*)
Desulfovibrio putealis DSM 16056 (*Deltaproteobacteria*)
Desulfovibrio sp. isolate SP109 91617 (*Deltaproteobacteria*)
Desulfovibrio sp. J2 (*Deltaproteobacteria*)
Desulfovibrio sp. L21-Syr-AB (*Deltaproteobacteria*)
Desulfovibrio sp. X2 (*Deltaproteobacteria*)
Desulfuromonadales bacterium GWC2_61_20 (*Deltaproteobacteria*)
Desulfuromonas sp. DDH964 (*Deltaproteobacteria*)
Desulfuromonas sp. WTL (*Deltaproteobacteria*)
Dethiobacter alkaliphilus AHT 1 (*Firmicutes-Clostridia*)
Elusimicrobia bacterium GWA2_64_40 (*Elusimicrobia*)
Elusimicrobia bacterium GWA2_69_24 (*Elusimicrobia*)
Elusimicrobia bacterium RIFOXYA2_FULL_40_6 (*Elusimicrobia*)
Elusimicrobia bacterium RIFOXYA2_FULL_47_53 (*Elusimicrobia*)
Elusimicrobia bacterium RIFOXYA12_FULL_49_49 (*Elusimicrobia*)
Elusimicrobia bacterium RIFOXYB1_FULL_48_9 (*Elusimicrobia*)
Elusimicrobia bacterium RIFOXYB2_FULL_46_23 (*Elusimicrobia*)

Elusimicrobia bacterium RIFOXYB2_FULL_48_7 (*Elusimicrobia*)*
Elusimicrobia bacterium RIFOXYB12_FULL_50_12 (*Elusimicrobia*)
Ethanoligenens harbinense YUAN-3 chromosome (*Firmicutes-Clostridia*)
Gammaproteobacteria bacterium isolate NP949 (*Gammaproteobacteria*)*
Geoalkalibacter ferrihydriticus DSM 17813 (*Deltaproteobacteria*)
Geobacter anodireducens SD-1 (*Deltaproteobacteria*)
Geobacter argillaceus ATCC BAA-1139 (*Deltaproteobacteria*)
Geobacter bemandjiensis Bem (*Deltaproteobacteria*)
Geobacter bremensis R1 (*Deltaproteobacteria*)
Geobacter daltonii (*Deltaproteobacteria*)
Geobacter metallireducens GS-15 (*Deltaproteobacteria*)
Geobacter metallireducens RCH3 (*Deltaproteobacteria*)
Geobacter pickeringii G13 DSM 17153 (*Deltaproteobacteria*)
Geobacter soli GSS01 (*Deltaproteobacteria*)
Geobacter sp. M18 (*Deltaproteobacteria*)
Geobacter sp. M21 (*Deltaproteobacteria*)
Geobacter sp. OR-1 (*Deltaproteobacteria*)
Geobacter sulfurreducens AM-1 (*Deltaproteobacteria*)
Geobacter sulfurreducens KN400 (*Deltaproteobacteria*)
Geobacter sulfurreducens PCA (*Deltaproteobacteria*)
Geobacter uraniumreducens Rf4 (*Deltaproteobacteria*)
Geopsychrobacter electrodiphilus DSM 16401 (*Deltaproteobacteria*)
Ignavibacteria bacterium RIFOXYA2 FULL 35 10 (*Chlorobi*)*
Kosmotoga pacifica SLHLJ1 (*Thermotogae*)
Lentisphaerae bacterium GWF2_57_35 (*Lentisphaerae*)
Lentisphaerae bacterium RIFOXYA12_64_32 (*Lentisphaerae*)
Lentisphaerae bacterium RIFOXYA12_FULL_48_11 (*Lentisphaerae*)
Lentisphaerae bacterium RIFOXYB12_FULL_65_16 (*Lentisphaerae*)
Lentisphaerae bacterium RIFOXYC12_FULL_60_16 (*Lentisphaerae*)
Marinilabilia salmonicolor MSL42 (*Bacteroidetes*)
Methanocella paludicola SNAE (*Methanomicrobia*)
Methanocella sp. RC-I (*Methanomicrobia*)
Methanococcoides methylutens DSM 2657 (*Methanomicrobia*)
Methanocorpusculum bavaricum DSM 4179 (*Methanomicrobia*)
Methanofollis liminatans GKZPZ DSM 4140 (*Methanomicrobia*)
Methanolobus profundi Mob M (*Methanomicrobia*)
Methanolobus psychrophilus R15 (*Methanomicrobia*)
Methanolobus tindarius DSM 2278 (*Methanomicrobia*)
Methanolobus vulcani PL 12M (*Methanomicrobia*)
Methanomassiliicoccales archaeon RumEn M1 Ga0117923 (*Thermoplasmata*)

Methanomassiliicoccus luminyensis B10 (*Thermoplasmata*)
Methanomethylovorans hollandica DSM 15978 (*Methanomicrobia*)
Methanoregula formicicum SMSP (*Methanomicrobia*)
Methanoregulaceae archaeon JGI M3C4D3-001-G22 (*Methanomicrobia*)
Methanospirillum hungatei JF-1 (*Methanomicrobia*)
Natronincola peptidivorans DSM 18979 (*Firmicutes-Clostridia*)
Nitrospina AB-629-B06 (*Nitrospina*)*
Nitrospina SCGC AAA288-L16 (*Nitrospina*)*
Nitospira bacterium SG8_3_2 (*Nitrospirae*)
Nitospira bacterium SG8_3 (*Nitrospirae*)
Nitospira bacterium SG8_35_1 (*Nitrospirae*)
Nitrospirae bacterium GWC2_56_14_2 (*Nitrospirae*)
Nitrospirae bacterium GWC2_56_14 (*Nitrospirae*)
Nitrospirae bacterium GWF2_44_13 (*Nitrospirae*)
Nitrospirae bacterium RBG_19FT_COMBO_55_12 (*Nitrospirae*)
Nitrospirae bacterium RIFOXYA2_FULL_44_9 (*Nitrospirae*)
Nitrospirae bacterium RIFOXYB2_FULL_43_5_partialB (*Nitrospirae*)
OMZ North Pacific GP0053648 LPjun08P16500mDRAFT (*Nitrospina*)*
OMZ North Pacific GP0053648 LPjun08P41300mDRAFT (*Nitrospina*)*
Pelobacter seleniigenes DSM 18267 (*Deltaproteobacteria*)
Pelobacteraceae bacterium GWC2_58_13 (*Deltaproteobacteria*)
Phycisphaerae bacterium SG8_4 (*Planctomyces*)
Pyrococcus furiosus COM1 DSM 3638 (*Thermococci*)
Smithella sp. F21 (*Deltaproteobacteria*)
Spirochaeta sp. JC202 (*Spirochaetes*)
Spirochaetes bacterium GWB1_27_13 (*Spirochaetes*)
Spirochaetes bacterium GWB1_36_13 (*Spirochaetes*)
Spirochaetes bacterium GWB1_60_80 (*Spirochaetes*)
Spirochaetes bacterium GWB1_66_5 (*Spirochaetes*)
Spirochaetes bacterium GWE2_31_10 (*Spirochaetes*)
Spirochaetes bacterium GWF1_31_7 (*Spirochaetes*)
Spirochaetes bacterium GWF1_41_5 (*Spirochaetes*)
Spirochaetes bacterium GWF1_49_6 (*Spirochaetes*)
Spirochaetes bacterium GWF1_51_8 (*Spirochaetes*)
Spirochaetes bacterium GWF1_60_12 (*Spirochaetes*)
Spirochaetes bacterium RBG_16_49_21 (*Spirochaetes*)
Spirochaetes bacterium RBG_16_67_19 (*Spirochaetes*)
Spirochaetes bacterium RIFOXYB1_FULL_32_8 (*Spirochaetes*)
Spirochaetes bacterium RIFOXYC1_FULL_54_7 (*Spirochaetes*)
Streptomyces sp. CNQ-509 (*Actinobacteria*)

Syntrophobacterales bacterium GWC2_56_13 (*Delta*proteobacteria)
Syntrophobacterales bacterium RBG_19FT_COMBO_59_10 (*Delta*proteobacteria)*
Syntrophobotulus glycolicus DSM_8271 (*Firmicutes-Clostridia*)
Syntrophorhabdus aromaticivorans UI (*Delta*proteobacteria)
Syntrophus aciditrophicus SB (*Delta*proteobacteria)
Syntrophus gentianae DSM 8423 (*Delta*proteobacteria)
Syntrophus sp. G20 Ga0063310 (*Delta*proteobacteria)
Theionarchaea archaeon DG-70-1 (*Theionarchaea*)
Thermococcus sp. EP1 (*Thermococci*)
Treponema sp. GWA1_62_8 (*Spirochaetes*)
Treponema sp. GBW1_62_6 (*Spirochaetes*)
uncultured *Desulfarculaceae* 191 (*Delta*proteobacteria)*

Supplementary Table 2. All forward and reverse primer oligonucleotide sequences from this study, including GC content (%), annealing temperature (Tm), whether the sequence has an exact match to reference library (Table S1) or amplicon library (Table S4) *hgcA* and *hgcB* sequences, and if the sequence is shared between reverse primers (shown in bold).

Sequence Name	Sequence 5' to 3'	% GC	Tm (°C)	Reference (Y/N)	Amplicon (Y/N)	Same As
ORNL-HgcAB-uni-F	AAYGTCTGGTGYGCNGCVGG	68.8	60.9 - 70.7	Y	Y	
ORNL-HgcAB-uni-32R	CAGGCNCCGCAYTCSATRCA	64.7	60.2 - 68.5	Y	Y	
ORNL-HgcAB-uni-R	CABGCNCCRCAYTCCATRCA	60	56.1 - 68.2	Y	Y	
ORNL-HgcAB-uni-F1	AACGTCTGGTGCGCAGCCGG	70	68.4	N	Y	
ORNL-HgcAB-uni-F2	AACGTCTGGTGCGCAGCGGG	70	68.4	Y	Y	
ORNL-HgcAB-uni-F3	AACGTCTGGTGCGCAGCAGG	65	66	N	Y	
ORNL-HgcAB-uni-F4	AACGTCTGGTGCGCCGCCGG	75	70.7	Y	Y	
ORNL-HgcAB-uni-F5	AACGTCTGGTGCGCCGCCGG	75	70.7	Y	Y	
ORNL-HgcAB-uni-F6	AACGTCTGGTGCGCCGCAGG	70	68.4	Y	Y	
ORNL-HgcAB-uni-F7	AACGTCTGGTGCGCTGCCGG	70	68.4	Y	Y	
ORNL-HgcAB-uni-F8	AACGTCTGGTGCGCTGCCGG	70	68.4	Y	Y	
ORNL-HgcAB-uni-F9	AACGTCTGGTGCGCTGCAGG	65	66	N	Y	
ORNL-HgcAB-uni-F10	AACGTCTGGTGCGCGGCCGG	75	70.7	Y	Y	
ORNL-HgcAB-uni-F11	AACGTCTGGTGCGCGGCCGG	75	70.7	Y	Y	
ORNL-HgcAB-uni-F12	AACGTCTGGTGCGCGGCCAGG	70	68.4	Y	Y	
ORNL-HgcAB-uni-F13	AACGTCTGGTGTGCAGCCGG	65	65.7	Y	Y	
ORNL-HgcAB-uni-F14	AACGTCTGGTGTGCAGCGGG	65	65.7	N	Y	
ORNL-HgcAB-uni-F15	AACGTCTGGTGTGCAGCAGG	60	63.3	N	Y	
ORNL-HgcAB-uni-F16	AACGTCTGGTGTGCCGCCGG	70	68.1	N	Y	
ORNL-HgcAB-uni-F17	AACGTCTGGTGTGCCGCCGG	70	68.1	N	Y	
ORNL-HgcAB-uni-F18	AACGTCTGGTGTGCCGCAGG	65	65.7	Y	Y	
ORNL-HgcAB-uni-F19	AACGTCTGGTGTGCTGCCGG	65	65.7	Y	Y	
ORNL-HgcAB-uni-F20	AACGTCTGGTGTGCTGCCGG	65	65.7	N	Y	
ORNL-HgcAB-uni-F21	AACGTCTGGTGTGCTGCAGG	60	63.3	N	Y	
ORNL-HgcAB-uni-F22	AACGTCTGGTGTGCCGCCGG	70	68.1	Y	Y	
ORNL-HgcAB-uni-F23	AACGTCTGGTGTGCCGCCGG	70	68.1	Y	Y	
ORNL-HgcAB-uni-F24	AACGTCTGGTGTGCCGCCAGG	65	65.7	N	Y	
ORNL-HgcAB-uni-F25	AATGTCTGGTGCAGCCGG	65	66.1	Y	Y	
ORNL-HgcAB-uni-F26	AATGTCTGGTGCAGCGGG	65	66.1	Y	Y	
ORNL-HgcAB-uni-F27	AATGTCTGGTGCAGCAGG	60	63.7	Y	Y	
ORNL-HgcAB-uni-F28	AATGTCTGGTGCAGCCGCCGG	70	68.5	Y	Y	
ORNL-HgcAB-uni-F29	AATGTCTGGTGCAGCCGCCGG	70	68.5	Y	Y	

ORNL-HgcAB-uni-F30	AATGTCTGGTGCGCCGCAGG	65	66.1	Y	Y	
ORNL-HgcAB-uni-F31	AATGTCTGGTGCCTGCCGG	65	66.1	Y	Y	
ORNL-HgcAB-uni-F32	AATGTCTGGTGCCTGCCGG	65	66.1	Y	Y	
ORNL-HgcAB-uni-F33	AATGTCTGGTGCCTGCAGG	60	63.7	Y	Y	
ORNL-HgcAB-uni-F34	AATGTCTGGTGCCTGCCGG	70	68.5	Y	Y	
ORNL-HgcAB-uni-F35	AATGTCTGGTGCCTGCCGG	70	68.5	Y	Y	
ORNL-HgcAB-uni-F36	AATGTCTGGTGCCTGCCGG	65	66.1	Y	Y	
ORNL-HgcAB-uni-F37	AATGTCTGGTGTGCAGCCGG	60	63.4	Y	Y	
ORNL-HgcAB-uni-F38	AATGTCTGGTGTGCAGCCGG	60	63.4	Y	Y	
ORNL-HgcAB-uni-F39	AATGTCTGGTGTGCAGCAGG	55	60.9	Y	Y	
ORNL-HgcAB-uni-F40	AATGTCTGGTGTGCCGCCGG	65	65.8	Y	Y	
ORNL-HgcAB-uni-F41	AATGTCTGGTGTGCCGCCGG	65	65.8	Y	Y	
ORNL-HgcAB-uni-F42	AATGTCTGGTGTGCCGCCAGG	60	63.4	Y	Y	
ORNL-HgcAB-uni-F43	AATGTCTGGTGTGCCTGCCGG	60	63.4	Y	Y	
ORNL-HgcAB-uni-F44	AATGTCTGGTGTGCCTGCCGG	60	63.4	Y	Y	
ORNL-HgcAB-uni-F45	AATGTCTGGTGTGCAGCAGG	55	60.9	N	Y	
ORNL-HgcAB-uni-F46	AATGTCTGGTGTGCCGCCGG	65	65.8	Y	Y	
ORNL-HgcAB-uni-F47	AATGTCTGGTGTGCCGCCGG	65	65.8	Y	Y	
ORNL-HgcAB-uni-F48	AATGTCTGGTGTGCCGCCAGG	60	63.4	Y	Y	
ORNL-HgcAB-uni-32R-1	CAGGCACCGCATTCCATACA	55	60.7	Y	Y	ORNL-HgcAB-uni-R-56
ORNL-HgcAB-uni-32R-2	CAGGCACCGCATTCCATGCA	60	64.1	Y	Y	ORNL-HgcAB-uni-R-8
ORNL-HgcAB-uni-32R-3	CAGGCACCGCATTCGATACA	55	60.8	N	Y	
ORNL-HgcAB-uni-32R-4	CAGGCACCGCATTCGATGCA	60	64.1	Y	Y	
ORNL-HgcAB-uni-32R-5	CAGGCACCGCACTCCATACA	60	62.5	Y	Y	ORNL-HgcAB-uni-R-80
ORNL-HgcAB-uni-32R-6	CAGGCACCGCACTCCATGCA	65	65.8	N	Y	ORNL-HgcAB-uni-R-32
ORNL-HgcAB-uni-32R-7	CAGGCACCGCACTCGATACA	60	62.6	N	Y	
ORNL-HgcAB-uni-32R-8	CAGGCACCGCACTCGATGCA	65	65.8	N	Y	
ORNL-HgcAB-uni-32R-9	CAGGCCTCCGCATTCCATACA	55	60.2	Y	Y	ORNL-HgcAB-uni-R-59
ORNL-HgcAB-uni-32R-10	CAGGCCTCCGCATTCCATGCA	60	63.5	Y	Y	ORNL-HgcAB-uni-R-11
ORNL-HgcAB-uni-32R-11	CAGGCCTCCGCATTCGATACA	55	60.2	N	Y	
ORNL-HgcAB-uni-32R-12	CAGGCCTCCGCATTCGATGCA	60	63.5	N	Y	
ORNL-HgcAB-uni-32R-13	CAGGCCTCCGCACTCCATACA	60	62	N	Y	ORNL-HgcAB-uni-R-83
ORNL-HgcAB-uni-32R-14	CAGGCCTCCGCACTCCATGCA	65	65.3	Y	Y	ORNL-HgcAB-uni-R-35
ORNL-HgcAB-uni-32R-15	CAGGCCTCCGCACTCGATACA	60	62	N	Y	
ORNL-HgcAB-uni-32R-16	CAGGCCTCCGCACTCGATGCA	65	65.3	N	Y	
ORNL-HgcAB-uni-32R-17	CAGGCCCGCATTCCATACA	60	62.6	Y	Y	ORNL-HgcAB-uni-R-53
ORNL-HgcAB-uni-32R-18	CAGGCCCGCATTCCATGCA	65	66	Y	Y	ORNL-HgcAB-uni-R-5
ORNL-HgcAB-uni-32R-19	CAGGCCCGCATTCGATACA	60	62.7	N	Y	
ORNL-HgcAB-uni-32R-20	CAGGCCCGCATTCGATGCA	65	65.9	Y	Y	

ORNL-HgcAB-uni-32R-21	CAGGCCCGCACTCCATACA	65	64.4	Y	Y	ORNL-HgcAB-uni-R-77
ORNL-HgcAB-uni-32R-22	CAGGCCCGCACTCCATGCA	70	67.7	Y	Y	ORNL-HgcAB-uni-R-29
ORNL-HgcAB-uni-32R-23	CAGGCCCGCACTCGATACA	65	64.4	N	Y	
ORNL-HgcAB-uni-32R-24	CAGGCCCGCACTCGATGCA	70	67.7	N	Y	
ORNL-HgcAB-uni-32R-25	CAGGCGCCGCACTCCATACA	60	63.5	Y	Y	ORNL-HgcAB-uni-R-50
ORNL-HgcAB-uni-32R-26	CAGGCGCCGCACTCCATGCA	65	66.8	Y	Y	ORNL-HgcAB-uni-R-2
ORNL-HgcAB-uni-32R-27	CAGGCGCCGCAATTGATACA	60	63.6	Y	Y	
ORNL-HgcAB-uni-32R-28	CAGGCGCCGCAATTGATGCA	65	66.8	Y	Y	
ORNL-HgcAB-uni-32R-29	CAGGCGCCGCACTCCATACA	65	65.3	Y	Y	ORNL-HgcAB-uni-R-74
ORNL-HgcAB-uni-32R-30	CAGGCGCCGCACTCCATGCA	70	68.5	Y	Y	ORNL-HgcAB-uni-R-26
ORNL-HgcAB-uni-32R-31	CAGGCGCCGCACTCGATACA	65	65.3	N	Y	
ORNL-HgcAB-uni-32R-32	CAGGCGCCGCACTCGATGCA	70	68.5	N	Y	
ORNL-HgcAB-uni-R-1	CATGCTCCGCACTCCATGCA	60	63.5	N	Y	
ORNL-HgcAB-uni-R-2	CAGGCCTCCGCACTCCATGCA	65	65.3	N	Y	ORNL-HgcAB-uni-32R-26
ORNL-HgcAB-uni-R-3	CACGCTCCGCACTCCATGCA	65	65.8	N	Y	
ORNL-HgcAB-uni-R-4	CATGCGCCGCACTCCATGCA	65	66.8	Y	Y	
ORNL-HgcAB-uni-R-5	CAGGCGCCGCACTCCATGCA	70	68.5	Y	Y	ORNL-HgcAB-uni-32R-18
ORNL-HgcAB-uni-R-6	CACGCGCCGCACTCCATGCA	70	69	Y	Y	
ORNL-HgcAB-uni-R-7	CATGCACCGCACTCCATGCA	60	64	N	Y	
ORNL-HgcAB-uni-R-8	CAGGCACCGCACTCCATGCA	65	65.8	N	Y	ORNL-HgcAB-uni-32R-26
ORNL-HgcAB-uni-R-9	CACGCACCGCACTCCATGCA	65	66.3	N	Y	
ORNL-HgcAB-uni-R-10	CATGCCCGCACTCCATGCA	65	65.9	Y	Y	
ORNL-HgcAB-uni-R-11	CAGGCCCGCACTCCATGCA	70	67.7	Y	Y	ORNL-HgcAB-uni-32R-10
ORNL-HgcAB-uni-R-12	CACGCCCGCACTCCATGCA	70	68.2	Y	Y	
ORNL-HgcAB-uni-R-13	CATGCTCCACACTCCATGCA	55	60.7	N	Y	
ORNL-HgcAB-uni-R-14	CAGGCCTCCACACTCCATGCA	60	62.5	Y	Y	
ORNL-HgcAB-uni-R-15	CACGCTCCACACTCCATGCA	60	63.1	N	Y	
ORNL-HgcAB-uni-R-16	CATGCGCCACACTCCATGCA	60	64	N	Y	
ORNL-HgcAB-uni-R-17	CAGGCGCCACACTCCATGCA	65	65.8	N	Y	
ORNL-HgcAB-uni-R-18	CACGCGCCACACTCCATGCA	65	66.3	N	Y	
ORNL-HgcAB-uni-R-19	CATGCACCAACACTCCATGCA	55	61.2	Y	Y	
ORNL-HgcAB-uni-R-20	CAGGCACCAACACTCCATGCA	60	63	N	Y	
ORNL-HgcAB-uni-R-21	CACGCACCAACACTCCATGCA	60	63.6	N	Y	
ORNL-HgcAB-uni-R-22	CATGCCCGACACTCCATGCA	60	63.1	N	Y	
ORNL-HgcAB-uni-R-23	CAGGCCCGACACTCCATGCA	65	64.9	Y	Y	
ORNL-HgcAB-uni-R-24	CACGCCCGACACTCCATGCA	65	65.5	N	Y	
ORNL-HgcAB-uni-R-25	CATGCTCCGCAATTGATGCA	55	61.7	Y	Y	
ORNL-HgcAB-uni-R-26	CAGGCCTCCGCAATTGATGCA	60	63.5	Y	Y	ORNL-HgcAB-uni-32R-30
ORNL-HgcAB-uni-R-27	CACGCTCCGCAATTGATGCA	60	64.1	N	Y	

ORNL-HgcAB-uni-R-28	CATGCCCGCATTCCATGCA	60	65	Y	Y	
ORNL-HgcAB-uni-R-29	CAGGCCCGCATTCCATGCA	65	66.8	Y	Y	ORNL-HgcAB-uni-32R-22
ORNL-HgcAB-uni-R-30	CACGCCCGCATTCCATGCA	65	67.3	N	Y	
ORNL-HgcAB-uni-R-31	CATGCACCGCATTCCATGCA	55	62.3	Y	Y	
ORNL-HgcAB-uni-R-32	CAGGCCACCGCATTCCATGCA	60	64.1	Y	Y	ORNL-HgcAB-uni-32R-6
ORNL-HgcAB-uni-R-33	CACGCCACCGCATTCCATGCA	60	64.6	Y	Y	
ORNL-HgcAB-uni-R-34	CATGCCCGCATTCCATGCA	60	64.2	Y	Y	
ORNL-HgcAB-uni-R-35	CAGGCCCGCATTCCATGCA	65	66	Y	Y	ORNL-HgcAB-uni-32R-14
ORNL-HgcAB-uni-R-36	CACGCCCGCATTCCATGCA	65	66.5	N	Y	
ORNL-HgcAB-uni-R-37	CATGCTCCACATTCCATGCA	50	58.9	Y	Y	
ORNL-HgcAB-uni-R-38	CAGGCTCCACATTCCATGCA	55	60.7	N	Y	
ORNL-HgcAB-uni-R-39	CACGCTCCACATTCCATGCA	55	61.3	N	Y	
ORNL-HgcAB-uni-R-40	CATGCCAACATTCCATGCA	55	62.3	N	Y	
ORNL-HgcAB-uni-R-41	CAGGCCAACATTCCATGCA	60	64.1	Y	Y	
ORNL-HgcAB-uni-R-42	CACGCCAACATTCCATGCA	60	64.6	Y	Y	
ORNL-HgcAB-uni-R-43	CATGCACCACATTCCATGCA	50	59.5	N	Y	
ORNL-HgcAB-uni-R-44	CAGGCACCACATTCCATGCA	55	61.3	N	Y	
ORNL-HgcAB-uni-R-45	CACGCACCACATTCCATGCA	55	61.9	N	Y	
ORNL-HgcAB-uni-R-46	CATGCCAACATTCCATGCA	55	61.3	N	Y	
ORNL-HgcAB-uni-R-47	CAGGCCAACATTCCATGCA	60	63.1	N	Y	
ORNL-HgcAB-uni-R-48	CACGCCAACATTCCATGCA	60	63.7	N	Y	
ORNL-HgcAB-uni-R-49	CATGCTCGCACTCCATACA	55	60.2	N	Y	
ORNL-HgcAB-uni-R-50	CAGGCTCCGCACTCCATACA	60	62	N	Y	ORNL-HgcAB-uni-32R-25
ORNL-HgcAB-uni-R-51	CACGCTCCGCACTCCATACA	60	62.6	N	Y	
ORNL-HgcAB-uni-R-52	CATGCCCGCACTCCATACA	60	63.5	N	Y	
ORNL-HgcAB-uni-R-53	CAGGCCCGCACTCCATACA	65	65.3	Y	Y	ORNL-HgcAB-uni-32R-17
ORNL-HgcAB-uni-R-54	CACGCCCGCACTCCATACA	65	65.8	Y	Y	
ORNL-HgcAB-uni-R-55	CATGCACCGCACTCCATACA	55	60.7	N	Y	
ORNL-HgcAB-uni-R-56	CAGGCACCGCACTCCATACA	60	62.5	Y	Y	ORNL-HgcAB-uni-32R-1
ORNL-HgcAB-uni-R-57	CACGCACCGCACTCCATACA	60	63.1	Y	Y	
ORNL-HgcAB-uni-R-58	CATGCCCGCACTCCATACA	60	62.6	N	Y	
ORNL-HgcAB-uni-R-59	CAGGCCCGCACTCCATACA	65	64.4	Y	Y	ORNL-HgcAB-uni-32R-9
ORNL-HgcAB-uni-R-60	CACGCCCGCACTCCATACA	65	65	N	Y	
ORNL-HgcAB-uni-R-61	CATGCTCCACACTCCATACA	50	57.3	Y	Y	
ORNL-HgcAB-uni-R-62	CAGGCTCCACACTCCATACA	55	59.1	Y	Y	
ORNL-HgcAB-uni-R-63	CACGCTCCACACTCCATACA	55	59.8	N	Y	
ORNL-HgcAB-uni-R-64	CATGCCAACACTCCATACA	55	60.7	N	Y	
ORNL-HgcAB-uni-R-65	CAGGCCAACACTCCATACA	60	62.5	N	Y	
ORNL-HgcAB-uni-R-66	CACGCCAACACTCCATACA	60	63.1	N	Y	

ORNL-HgcAB-uni-R-67	CATGCACCACACTCCATACA	50	57.9	Y	Y	
ORNL-HgcAB-uni-R-68	CAGGCACCACACTCCATACA	55	59.7	N	Y	
ORNL-HgcAB-uni-R-69	CACGCACCACACTCCATACA	55	60.3	N	Y	
ORNL-HgcAB-uni-R-70	CATGCCAACACTCCATACA	55	59.7	Y	Y	
ORNL-HgcAB-uni-R-71	CAGGCCAACACTCCATACA	60	61.6	Y	Y	
ORNL-HgcAB-uni-R-72	CACGCCAACACTCCATACA	60	62.2	N	Y	
ORNL-HgcAB-uni-R-73	CATGCTCCGCATTCCATACA	50	58.4	Y	Y	
ORNL-HgcAB-uni-R-74	CAGGCTCCGCATTCCATACA	55	60.2	Y	Y	ORNL-HgcAB-uni-32R-29
ORNL-HgcAB-uni-R-75	CACGCTCCGCATTCCATACA	55	60.8	N	Y	
ORNL-HgcAB-uni-R-76	CATGCGCCGCATTCCATACA	55	61.8	N	Y	
ORNL-HgcAB-uni-R-77	CAGGCGCCGCATTCCATACA	60	63.5	Y	Y	ORNL-HgcAB-uni-32R-21
ORNL-HgcAB-uni-R-78	CACGCGCCGCATTCCATACA	60	64.1	Y	Y	
ORNL-HgcAB-uni-R-79	CATGCACCGCATTCCATACA	50	59	Y	Y	
ORNL-HgcAB-uni-R-80	CAGGCACCGCATTCCATACA	55	60.7	Y	Y	ORNL-HgcAB-uni-32R-5
ORNL-HgcAB-uni-R-81	CACGCACCGCATTCCATACA	55	61.4	Y	Y	
ORNL-HgcAB-uni-R-82	CATGCCCGCATTCCATACA	55	60.8	N	Y	
ORNL-HgcAB-uni-R-83	CAGGCCCGCATTCCATACA	60	62.6	Y	Y	ORNL-HgcAB-uni-32R-13
ORNL-HgcAB-uni-R-84	CACGCCCGCATTCCATACA	60	63.2	Y	Y	
ORNL-HgcAB-uni-R-85	CATGCTCCACATTCCATACA	45	55.5	Y	Y	
ORNL-HgcAB-uni-R-86	CAGGCTCCACATTCCATACA	50	57.3	Y	Y	
ORNL-HgcAB-uni-R-87	CACGCTCCACATTCCATACA	50	58	N	Y	
ORNL-HgcAB-uni-R-88	CATGCCACATTCCATACA	50	59	Y	Y	
ORNL-HgcAB-uni-R-89	CAGGCCACATTCCATACA	55	60.7	Y	Y	
ORNL-HgcAB-uni-R-90	CACGCCACATTCCATACA	55	61.4	N	Y	
ORNL-HgcAB-uni-R-91	CATGCACCAATTCCATACA	45	56.1	Y	Y	
ORNL-HgcAB-uni-R-92	CAGGCACCAATTCCATACA	50	57.9	N	Y	
ORNL-HgcAB-uni-R-93	CACGCACCAATTCCATACA	50	58.6	N	Y	
ORNL-HgcAB-uni-R-94	CATGCCACATTCCATACA	50	57.9	Y	Y	
ORNL-HgcAB-uni-R-95	CAGGCCACATTCCATACA	55	59.7	N	Y	
ORNL-HgcAB-uni-R-96	CACGCCACATTCCATACA	55	60.4	N	Y	

Supplementary Table 3. Environmental samples used for *hgcAB* clone libraries. Unless otherwise designated, *hgcAB* clone sequences were amplified with primer set ORNL-HgcAB-uni-F/ORNL-HgcAB-uni-32R. The number of clones are those that passed quality filtering criteria and were classified as *hgcAB*. The environmental clone hgcA sequences from this study are publicly available under the NCBI GenBank accession numbers MT122211 - MT122744.

Clone Sample IDs	Sample Site	Environment Type	Sample collection (depth, cm)	Sample collection (date)	Location	Clones (#)
Spruce and Peatland Responses Under Changing Environments (SPRUCE) (Iversen et al., 2014)		Boreal spruce and peatland sediments	From surface (0 cm) to 255 cm depth		USDA Forest service Marcell Experiment Forest (Grand Rapids, MN)	232
4	2012-10-T-Hol	Treed Hollow	-30	2012-08-14	S1-Bog, Plot #10	2
5	2012-10-T-Hol	Treed Hollow	-40	2012-08-14	S1-Bog, Plot #10	5
6	2012-10-T-Hol	Treed Hollow	-50	2012-08-14	S1-Bog, Plot #10	5
7	2012-10-T-Hol	Treed Hollow	-60	2012-08-14	S1-Bog, Plot #10	5
8	2012-10-T-Hol	Treed Hollow	-70	2012-08-14	S1-Bog, Plot #10	3
9	2012-10-T-Hol	Treed Hollow	-80	2012-08-14	S1-Bog, Plot #10	5
10	2012-10-T-Hol	Treed Hollow	-90	2012-08-14	S1-Bog, Plot #10	2
11	2012-10-T-Hol	Treed Hollow	-100	2012-08-14	S1-Bog, Plot #10	5
12	2012-10-T-Hol	Treed Hollow	-125	2012-08-14	S1-Bog, Plot #10	3
13	2012-10-T-Hol	Treed Hollow	-150	2012-08-14	S1-Bog, Plot #10	4
15	2012-10-T-Hol	Treed Hollow	-200	2012-08-14	S1-Bog, Plot #10	5
16	2012-10-T-Hol	Treed Hollow	-225	2012-08-14	S1-Bog, Plot #10	7
17	2012-10-T-Hol	Treed Hollow	-255	2012-08-14	S1-Bog, Plot #10	5
20	2012-10-T-Hum	Treed Hummock	+10	2012-08-14	S1-Bog, Plot #10	3
21	2012-10-T-Hum	Treed Hummock	0	2012-08-14	S1-Bog, Plot #10	2
22	2012-10-T-Hum	Treed Hummock	-10	2012-08-14	S1-Bog, Plot #10	5
23	2012-10-T-Hum	Treed Hummock	-20	2012-08-14	S1-Bog, Plot #10	4
24	2012-10-T-Hum	Treed Hummock	-30	2012-08-14	S1-Bog, Plot #10	4
25	2012-10-T-Hum	Treed Hummock	-40	2012-08-14	S1-Bog, Plot #10	5
26	2012-10-T-Hum	Treed Hummock	-50	2012-08-14	S1-Bog, Plot #10	4
27	2012-10-T-Hum	Treed Hummock	-60	2012-08-14	S1-Bog, Plot #10	5
28	2012-10-T-Hum	Treed Hummock	-70	2012-08-14	S1-Bog, Plot #10	6
29	2012-10-T-Hum	Treed Hummock	-80	2012-08-14	S1-Bog, Plot #10	5
30	2012-10-T-Hum	Treed Hummock	-90	2012-08-14	S1-Bog, Plot #10	5
31	2012-10-T-Hum	Treed Hummock	-100	2012-08-14	S1-Bog, Plot #10	4
32	2012-10-T-Hum	Treed Hummock	-125	2012-08-14	S1-Bog, Plot #10	4
33	2012-10-T-Hum	Treed Hummock	-150	2012-08-14	S1-Bog, Plot #10	4
34	2012-10-T-Hum	Treed Hummock	-175	2012-08-14	S1-Bog, Plot #10	5
39	2012-6-T-Hol	Treed Hollow	-40	2012-08-14	S1-Bog, Plot #6	2
40	2012-6-T-Hol	Treed Hollow	-50	2012-08-14	S1-Bog, Plot #6	4
41	2012-6-T-Hol	Treed Hollow	-60	2012-08-14	S1-Bog, Plot #6	4
42	2012-6-T-Hol	Treed Hollow	-70	2012-08-14	S1-Bog, Plot #6	5
43	2012-6-T-Hol	Treed Hollow	-80	2012-08-14	S1-Bog, Plot #6	4
44, 44H, 44L	2012-6-T-Hol	Treed Hollow	-90	2012-08-14	S1-Bog, Plot #6	10
45H	2012-6-T-Hol	Treed Hollow	-100	2012-08-14	S1-Bog, Plot #6	4
46, 46-4, 46-7	2012-6-T-Hol	Treed Hollow	-125	2012-08-14	S1-Bog, Plot #6	5

47, 47-2, 47H, 47L	2012-6-T-Hol	Treed Hollow	-150	2012-08-14	S1-Bog, Plot #6	12
48-5, 48-17	2012-6-T-Hol	Treed Hollow	-175	2012-08-14	S1-Bog, Plot #6	6
49	2012-6-T-Hol	Treed Hollow	-200	2012-08-14	S1-Bog, Plot #6	5
53, 53-17	2012-6-T-Hum	Treed Hummock	-10	2012-08-14	S1-Bog, Plot #6	5
54, 54H	2012-6-T-Hum	Treed Hummock	-30	2012-08-14	S1-Bog, Plot #6	5
55	2012-6-T-Hum	Treed Hummock	-40	2012-08-14	S1-Bog, Plot #6	6
56, 56H	2012-6-T-Hum	Treed Hummock	-50	2012-08-14	S1-Bog, Plot #6	3
57,57H	2012-6-T-Hum	Treed Hummock	-60	2012-08-14	S1-Bog, Plot #6	4
58, 58H	2012-6-T-Hum	Treed Hummock	-70	2012-08-14	S1-Bog, Plot #6	6
59,59H	2012-6-T-Hum	Treed Hummock	-80	2012-08-14	S1-Bog, Plot #6	4
60	2012-6-T-Hum	Treed Hummock	-90	2012-08-14	S1-Bog, Plot #6	5
61	2012-6-T-Hum	Treed Hummock	-100	2012-08-14	S1-Bog, Plot #6	5
62	2012-6-T-Hum	Treed Hummock	-125	2012-08-14	S1-Bog Plot #6	3
63	2012-6-T-Hum	Treed Hummock	-150	2012-08-14	S1-Bog, Plot #6	4
SPRUCE1 (OP1 ⁺ , NP1)	Equimolar mixture of gDNA from SPRUCE samples 16, 34, 40, and 57					58 (OP1 ⁺) , 65 (NP1)
Sandy Creek (BRL) (Ndu et al., 2018)	Freshwater stream sediment	Surface grab sample	2017-03-03	Durham, NC	19	
New Horizon sediment (HGC5, OP3 ⁺ , NP3)	Freshwater sediments	Surface grab sample (0 cm)	2017-09-21 (HGC5); 2018-01-19 (NP3, OP3)	East Fork Poplar Creek, Oak Ridge, TN	3 (HGC 5), 42 (OP3 ⁺), 61 (NP3)	
New Horizon periphyton (HGC2, Y117) (Olsen et al., 2016)	Freshwater periphyton biomass	Collected from rocks in stream	2016-08-23 (Y117) And 2017-09-21(HGC2)	East Fork Poplar Creek, Oak Ridge, TN	1 (HGC 2), 14 (Y117)	
Mesohaline tidal salt marsh sediment (GC) (Mitchell and Gilmour, 2008)	Marsh sediment	Top 15 cm	2015-09-30	Rhode River, Edgewater, MD	12	
Yanwuping Rice Paddy (D118) (Vishnivetskaya et al., 2018)	Rice paddy soils	5 cm depth	2011-07	Guizhou, China	38	

⁺*hgcAB* clone sequences from primer set ORNL-HgcAB-uni-F/ORNL-HgcAB-uni-R

Supplementary Table 4. Miseq *hgcAB* amplicon sequencing libraries for environmental, mock, and spiked communities. The environmental and mock community *hgcAB* amplicon raw sequence files are publicly available at the NCBI SRA accession (PRJNA608965).

Sample type	Sample environment	Sample ID	Amplicon library	Replicates + (#)	Reverse primer	PCR cycles	Amplicons (#)
Environmental	Tidal salt marsh sediment (1064) New Horizon (NH) surface sediment	1064	1064_Alt35	1	ORNL-HgcAB-uni-32R	x35	58374
			1064_Uni35	1	ORNL-HgcAB-uni-R	x35	41218
		NH1	NH1_Alt35	1	ORNL-HgcAB-uni-32R	x35	43828
			NH1_Uni35	1	ORNL-HgcAB-uni-R	x35	62533
		NH2	NH2_Alt35	1	ORNL-HgcAB-uni-32R	x35	63434
			NH2_Uni35	1	ORNL-HgcAB-uni-R	x35	69702
		NH3	NH3_Alt35	1	ORNL-HgcAB-uni-32R	x35	96455
			NH3_Uni35	1	ORNL-HgcAB-uni-R	x35	61379
		NH4	NH4_Alt30	1	ORNL-HgcAB-uni-32R	x30	58452
			NH4_Uni30	1	ORNL-HgcAB-uni-R	x30	46964
		NH5	NH5_Alt30	1	ORNL-HgcAB-uni-32R	x30	80990
			NH5_Uni30	1	ORNL-HgcAB-uni-R	x30	44168
		NH6	NH6_Alt30	1	ORNL-HgcAB-uni-32R	x30	24519
			NH6_Uni30	1	ORNL-HgcAB-uni-R	x30	20762
		NH-H	NH-H-Alt30	4	ORNL-HgcAB-uni-32R	X30	68046
		NH-I	NH-I-Alt30	1	ORNL-HgcAB-uni-32R	X30	22275
		NH-J	NH-J-Alt30	1	ORNL-HgcAB-uni-32R	X30	40817
		NH-K	NH-K-Alt30	4	ORNL-HgcAB-uni-32R	X30	102968
Environmental + spike	1064 + mock community 1 spike	1064_combo 1	m1064_1combo_Alt30	4	ORNL-HgcAB-uni-32R	x30	103803
			m1064_1combo_Alt35	2	ORNL-HgcAB-uni-32R	x35	93182
			m1064_1combo_Uni30	5	ORNL-HgcAB-uni-R	x30	231095
			m1064_1combo_Uni35	2	ORNL-HgcAB-uni-R	x35	141747
	1064 + mock community 2 spike	1064_combo 2	m1064_2combo_Alt30	5	ORNL-HgcAB-uni-32R	x30	133057
			m1064_2combo_Alt35	6	ORNL-HgcAB-uni-32R	x35	656352
			m1064_2combo_Uni30	5	ORNL-HgcAB-uni-R	x30	88372
			m1064_2combo_Uni35p1	5	ORNL-HgcAB-uni-R	x35	290878
Mock community	Mock community 1	combo1	m1combo_alt30	5	ORNL-HgcAB-uni-32R	x30	284603
	Mock community 1		m1combo_uni30	6	ORNL-HgcAB-uni-R	x30	235704
	Mock community 2	combo2	m2combo_Alt30p1	5	ORNL-HgcAB-uni-32R	x30	546866
	Mock community 2		m2combo_Uni30p1n1	6	ORNL-HgcAB-uni-R	x30	322774

+ total number of biological and sequencing replicates included in analyses

1 **Supplementary Table 5.** Occurrence of reverse oligo sequences in reference *hgcAB* sequences
 2 (*n* = 239) and percent occurrence in environmental *hgcAB* clones (*n* = 369), and amplicons (*n* =
 3 233934) amplified by ORNL-HgcAB-uni-F and ORNL-HgcAB-uni-32R.

ORNL-HgcAB-uni-32R oligo	Primer Sequence	Reference <i>hgcB</i> database (<i>n</i> =239)			% in environmental clones (<i>n</i> = 369)	% in environmental amplicons (<i>n</i> = 233934)
		0-mismatch	1-mismatch	2-mismatches		
1	CAGGCACCGCATTCCATACA	3	18	82	0.5	0.6
2	CAGGCACCGCATTCCATGCA	4	46	126	3.5	2.4
3	CAGGCACCGCATTGATACA	0	7	35	0.8	1.2
4	CAGGCACCGCATTGATGCA	2	12	58	4.6	3.3
5	CAGGCACCGCACTCCATACA	1	13	84	0.0	0.9
6	CAGGCACCGCACTCCATGCA	0	51	118	0.5	2.5
7	CAGGCACCGCACTCGATACA	0	1	21	0.0	1.3
8	CAGGCACCGCACTCGATGCA	0	4	60	1.4	4.2
9	CAGGCTCCGCATTCCATACA	1	20	82	0.0	0.6
10	CAGGCTCCGCATTCCATGCA	6	41	126	4.3	2.8
11	CAGGCTCCGCATTGATACA	0	5	32	0.5	1.2
12	CAGGCTCCGCATTGATGCA	0	11	55	7.6	4.3
13	CAGGCTCCGCACTCCATACA	0	11	89	0.5	0.8
14	CAGGCTCCGCACTCCATGCA	1	54	109	3.3	2.8
15	CAGGCTCCGCACTCGATACA	0	0	17	0.5	1.3
16	CAGGCTCCGCACTCGATGCA	0	1	61	3.3	5.0
17	CAGGCCCGCATTCCATACA	4	27	108	0.0	0.7
18	CAGGCCCGCATTCCATGCA	13	71	129	4.6	2.5
19	CAGGCCCGCATTGATACA	0	7	40	0.8	1.2
20	CAGGCCCGCATTGATGCA	2	19	79	5.1	3.9
21	CAGGCCCGCACTCCATACA	4	39	102	0.0	0.9
22	CAGGCCCGCACTCCATGCA	22	69	123	2.4	2.9
23	CAGGCCCGCACTCGATACA	0	4	44	2.2	1.5
24	CAGGCCCGCACTCGATGCA	0	24	77	3.5	5.3
25	CAGGCGCCGCATTCCATACA	1	33	108	2.7	1.1
26	CAGGCGCCGCATTCCATGCA	14	70	133	10.3	5.8
27	CAGGCGCCGCATTGATACA	1	4	45	4.3	2.5
28	CAGGCGCCGCATTGATGCA	1	21	84	10.6	10.0
29	CAGGCGCCGCACTCCATACA	3	35	99	0.5	1.5
30	CAGGCGCCGCACTCCATGCA	22	66	120	10.0	6.9
31	CAGGCGCCGCACTCGATACA	0	6	38	3.3	3.1
32	CAGGCGCCGCACTCGATGCA	0	23	79	7.3	15.1
	% of sequences recovered	56.3%	96.9%	100%	84.4%	100%

5 **Supplementary Table 6.** Theoretical binding efficiencies of reverse primers

Primer Version	In silico binding to <i>hgcAB</i> reference database (n = 239)								In silico binding to Ferredoxin database (n = 14,161)			
	0 mismatches				2 mismatches allowed				0 mismatches		2 mismatches	
	D*.	F*.	M*.	All*	D*.	F*.	M*.	All*	<i>hgcB</i>	Non- <i>hgcB</i>	<i>hgcB</i>	Non- <i>hgcB</i>
ORNL-HgcAB-uni-32R	0.71	0.26	0.38	0.50	0.98	0.94	0.88	0.98	0.61	0	1.00	0.04
ORNL-HgcAB-uni-R	0.88	0.45	0.88	0.75	0.98	1.00	0.88	0.98	0.90	0	1.00	0.03

6 *Theoretical binding efficiencies (%) of each reverse primer to a reference database of 239 *hgcB* nucleotide
 7 sequences, including matches to *Delta proteobacteria* (D), *Firmicutes* (F), *Methanomicrobia* (M), and to a 4Fe-4S
 8 ferredoxin reference database that contained 88 *hgcB* sequences and 14,1073 non-*hgcB* sequences.
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 10
 11

12 **Supplementary Table 7.** All PCR conditions tested for optimal primer to template ratio for
 13 amplification of *hgcAB* from environmental samples.
 14

Condition	Template concentration (final)	Primer concentration (final)	Reaction Volume	Primer Set
A ⁺	0.5 ng/μl	1.0 μM	20 μl	1. ORNL-HgcAB-uni-F & ORNL-HgcAB-uni-R 2. ORNL-HgcAB-uni-F & ORNL-HgcAB-32-R 3. ORNL-HgcAB-uni-F & Equimolar 26 4. ORNL-HgcAB-uni-F & Equimolar 32
B*	0.5 ng/μl	0.5 μM	20 μl	1. ORNL-HgcAB-uni-F & ORNL-HgcAB-uni-R 2. ORNL-HgcAB-uni-F & ORNL-HgcAB-32-R 3. ORNL-HgcAB-uni-F & Equimolar 26 4. ORNL-HgcAB-uni-F & Equimolar 32
C	0.002 ng/μl	0.5 μM	50 μl	1. ORNL-HgcAB-uni-F & ORNL-HgcAB-uni-R 2. ORNL-HgcAB-uni-F & ORNL-HgcAB-32-R 3. ORNL-HgcAB-uni-F & Equimolar 26 4. ORNL-HgcAB-uni-F & Equimolar 32
D	0.002 ng/μl	1.0 μM	50 μl	1. ORNL-HgcAB-uni-F & ORNL-HgcAB-uni-R 2. ORNL-HgcAB-uni-F & ORNL-HgcAB-32-R 3. ORNL-HgcAB-uni-F & Equimolar 26 4. ORNL-HgcAB-uni-F & Equimolar 32
E	0.02 ng/μl	1.0 μM	50 μl	1. ORNL-HgcAB-uni-F & ORNL-HgcAB-uni-R 2. ORNL-HgcAB-uni-F & ORNL-HgcAB-32-R 3. ORNL-HgcAB-uni-F & Equimolar 26 4. ORNL-HgcAB-uni-F & Equimolar 32

15 [†]Condition from Christensen et al. (2016)

16 *Condition used to amplify *hgcAB* for cloning experiment

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