Figure S1:

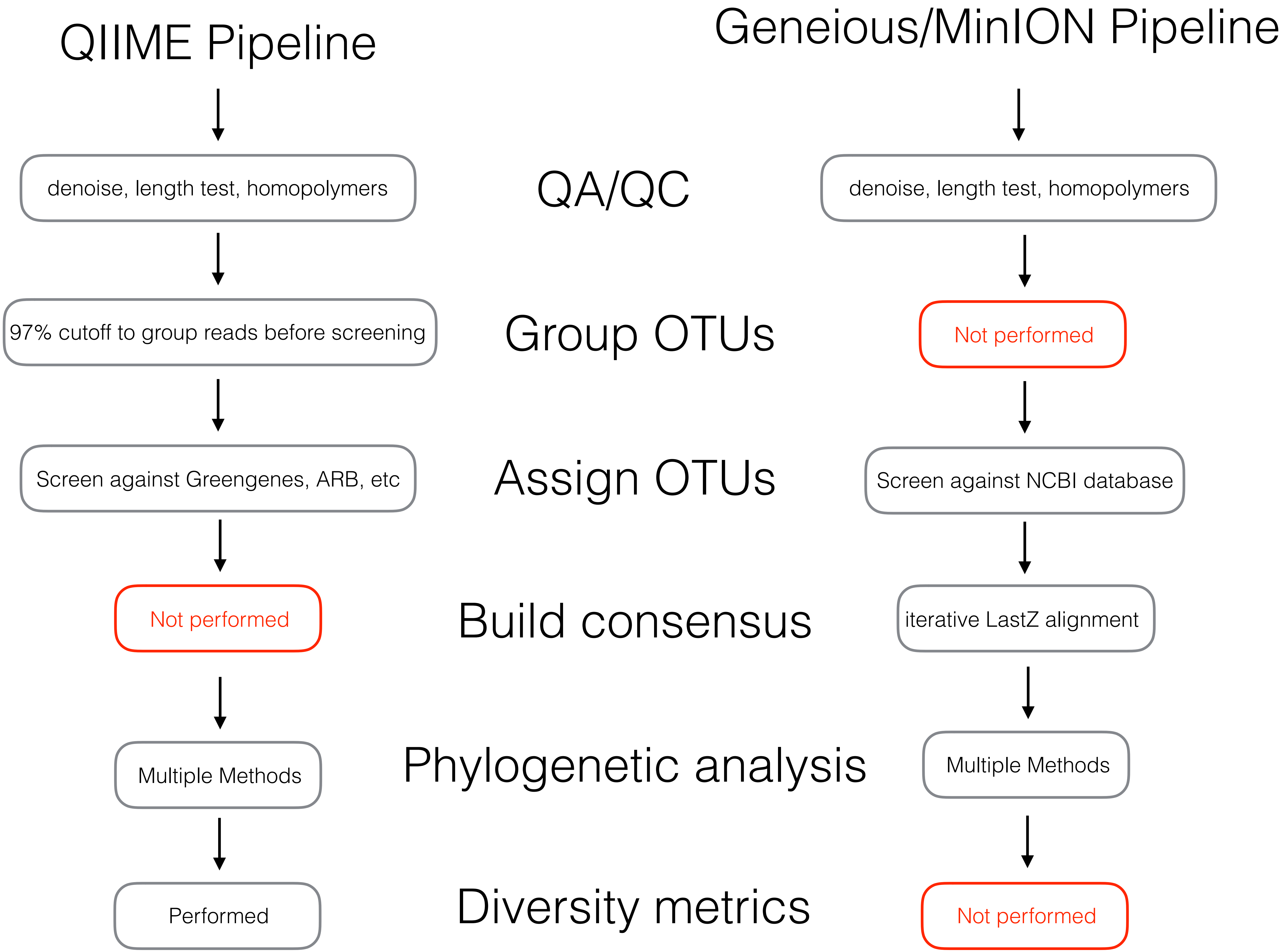
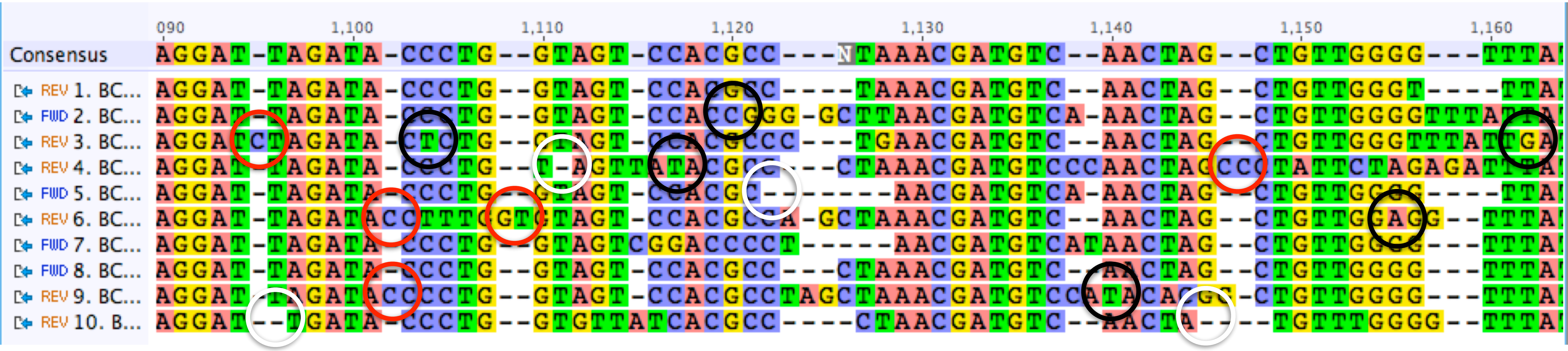

QIIME Pipeline
Geneious/MinION Pipeline
denoise, length test, homopolymers
QA/QC
denoise, length test, homopolymers
97% cutoff to group reads before screening
Group OTUs
Not performed
Screen against Greengenes, ARB, etc
Assign OTUs
Screen against NCBI database
Not performed
Build consensus
iterative LastZ alignment
Multiple Methods
Phylogenetic analysis
Multiple Methods
Performed
Diversity metrics
Not performed

Figure S2:



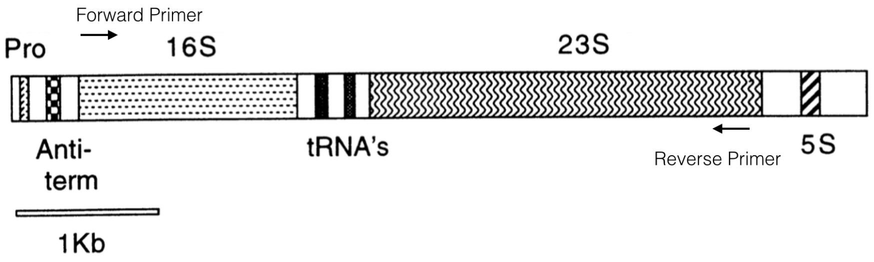
```
            090       1,100     1,110     1,120     1,130     1,140     1,150     1,160
Consensus   AGGAT-TAGATA-CCCTG--GTAGT-CCACGCC---NTAAACGATGTC--AACTAG--CTGTTGGGG---TTTA
REV 1. BC... AGGAT-TAGATA-CCCTG--GTAGT-CCACGCC----TAAACGATGTC--AACTAG--CTGTTGGGT----TTA
FWD 2. BC... AGGAT-TAGATA-CCCTG--GTAGT-CCACCGGG-GCTTAACGATGTCA-AACTAG--CTGTTGGGGTTTATTA
REV 3. BC... AGGATCTAGATA-CTCTG--GTAGT-CCACGCCC---TGAACGATGTC--AACTAG--CTGTTGGGTTTATTGA
REV 4. BC... AGGAT-TAGATA-CCCTG--T-AGTTATACGCC---CTAAACGATGTCCCAACTAGCCCTATTCTAGAGATTTA
FWD 5. BC... AGGAT-TAGATA-CCCTG--GTAGT-CCACGC-------AACGATGTCA-AACTAG--CTGTTGGGG----TTA
REV 6. BC... AGGAT-TAGATACCTTTGGTGTAGT-CCACGCCA-GCTAAACGATGTC--AACTAG--CTGTTGGAGG--TTTA
FWD 7. BC... AGGAT-TAGATA-CCCTG--GTAGTCGGACCCCT-----AACGATGTCATAACTAG--CTGTTGGGG---TTTA
FWD 8. BC... AGGAT-TAGATA-CCCTG--GTAGT-CCACGCC---CTAAACGATGTC--AACTAG--CTGTTGGGG---TTTA
REV 9. BC... AGGAT-TAGATACCCCTG--GTAGT-CCACGCCTAGCTAAACGATGTCCATACACGG-CTGTTGGGG---TTTA
REV 10. B... AGGAT--TGATA-CCCTG--GTGTTATCACGCC----CTAACGATGTC--AACTA----TGTTTGGGG--TTTA
```

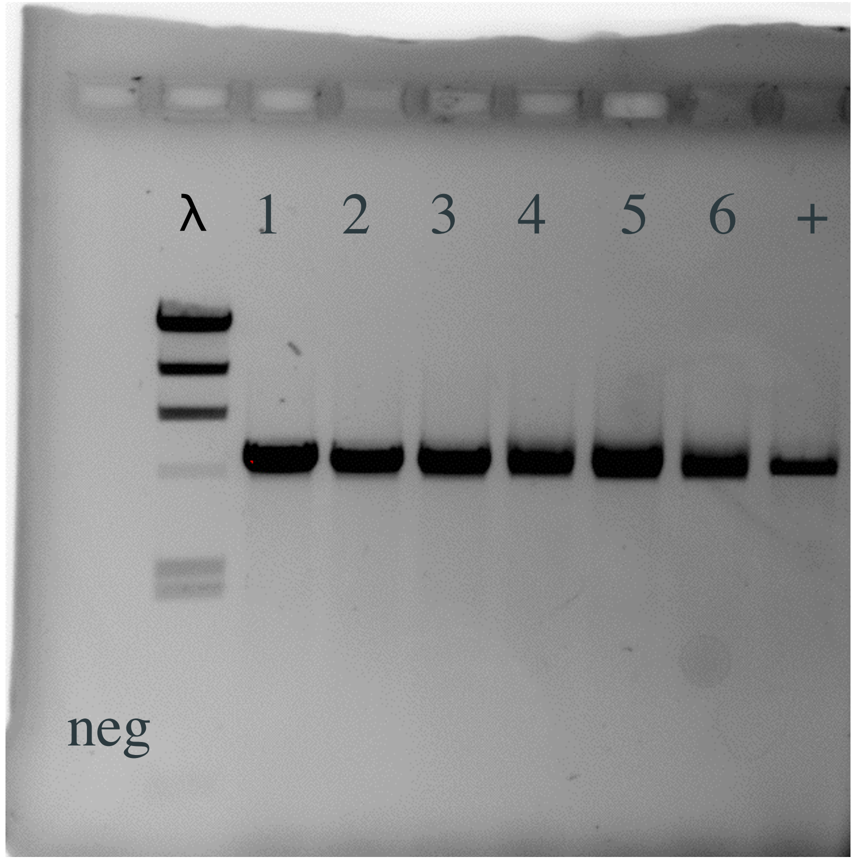
Insertions (red circle), deletions (white circle), and miscalled bases (black circle).

The consensus sequence is shown on the top row.

Figure S3:

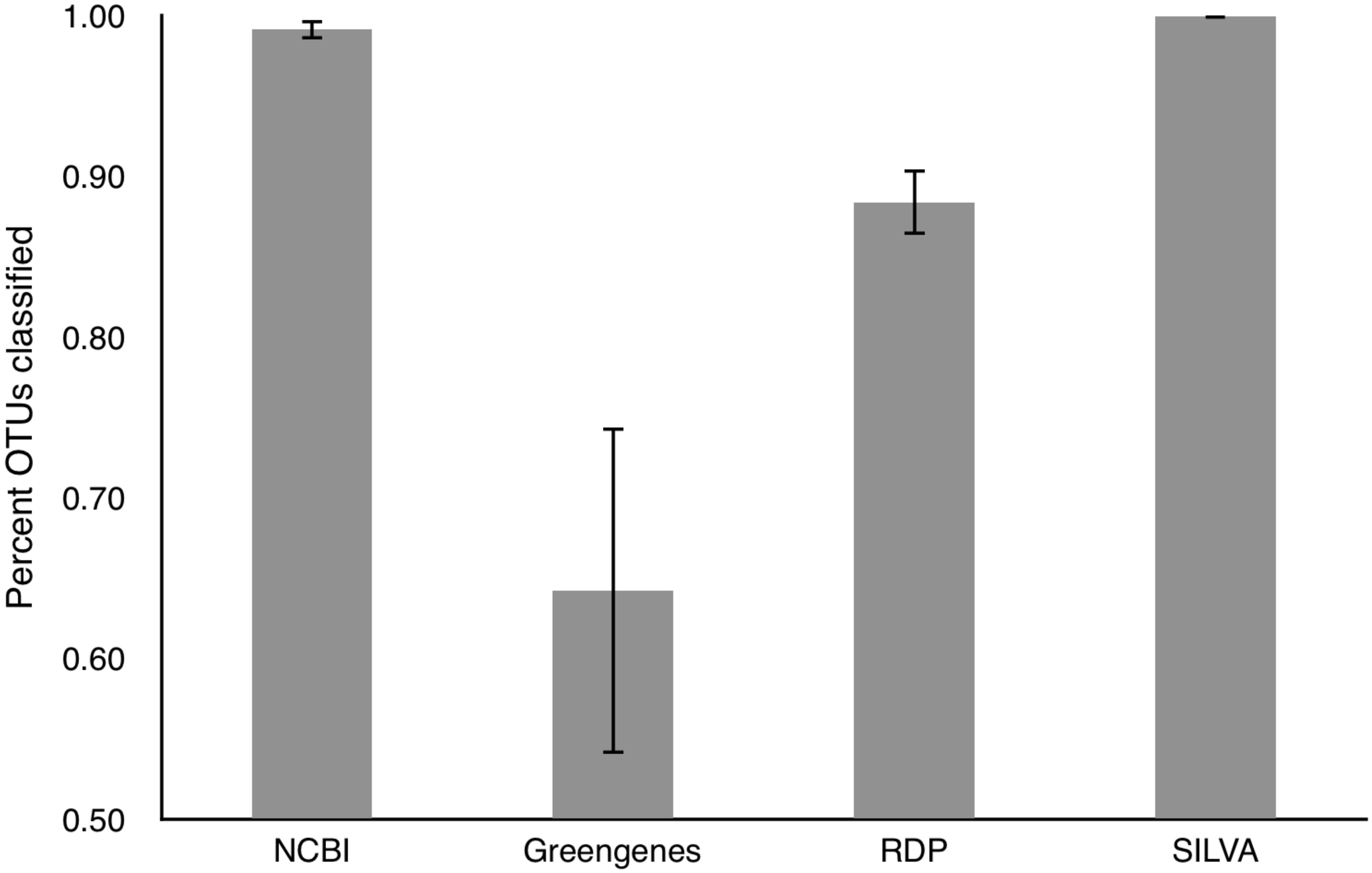
Schematic of a bacterial rRNA operon





The lanes indicate the various barcoded samples, the PCR negative (neg) and positive (+) controls, and lambda molecular weight standard.

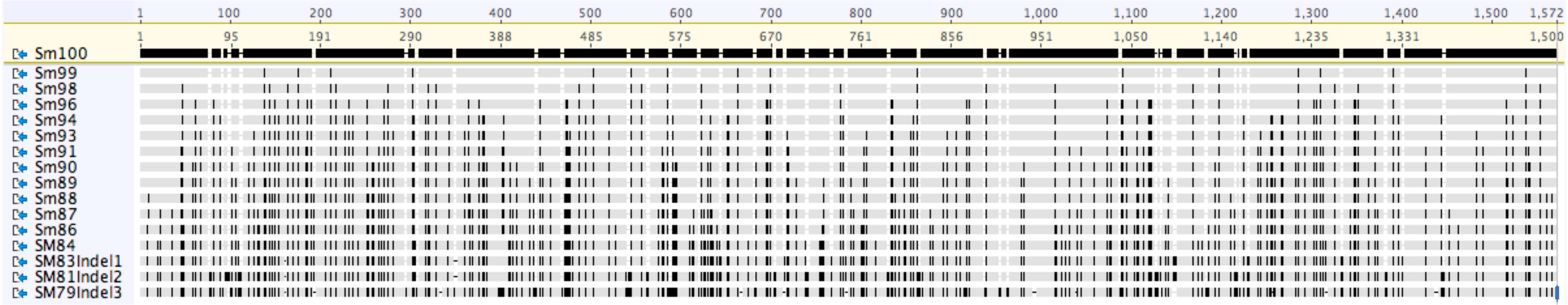
Figure S4:



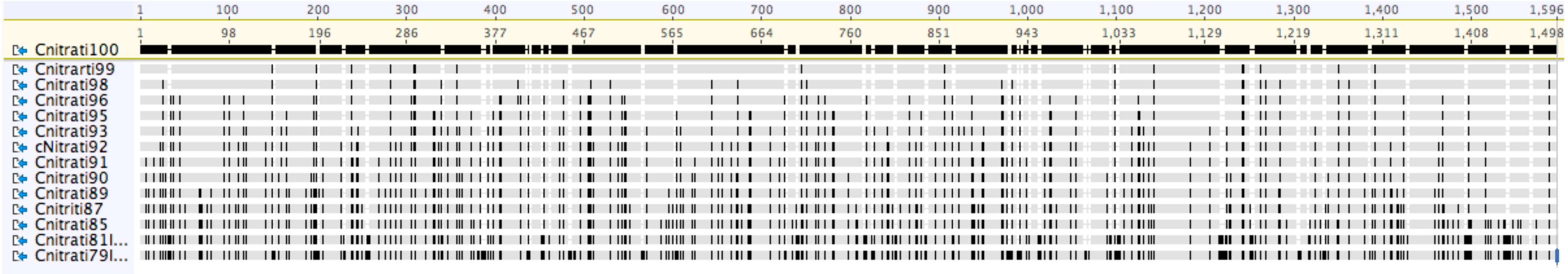
Unclassified OTUs are either defined as unclassified in the database or <70% identity over 500 bp. Data represents averages for the various biological replicates and the error bars are the standard deviation.

Figure S5:

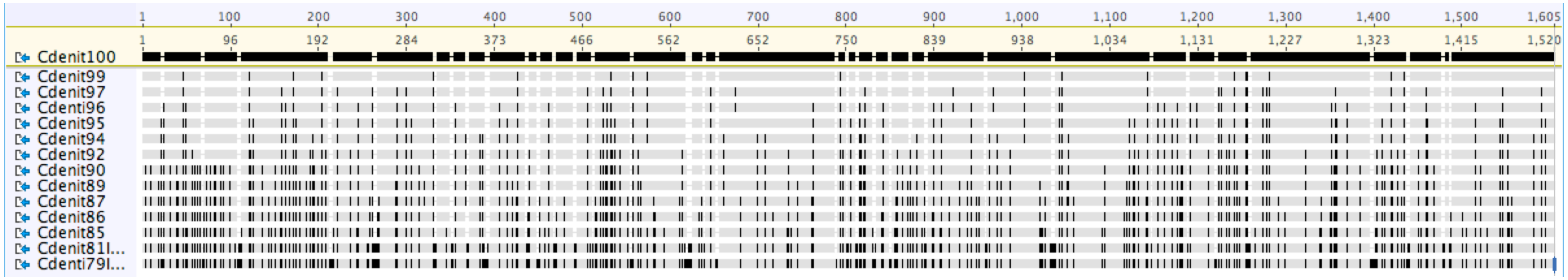
Stenotrophomonas maltophilia (100-79% similarity)



Comomonas nitrativorans (100-79% similarity)



Comomonas denitrificans (100-79% similarity)



Random substitutions and insertions/deletions were created in three 16S rRNA genes to generate 12-13 sequences between 79-100% identity. The positions of the substitutions are indicated below by the black lines. All sequences were then screened in Geneious and the results are shown in Additional Table 2.

Figure S6:

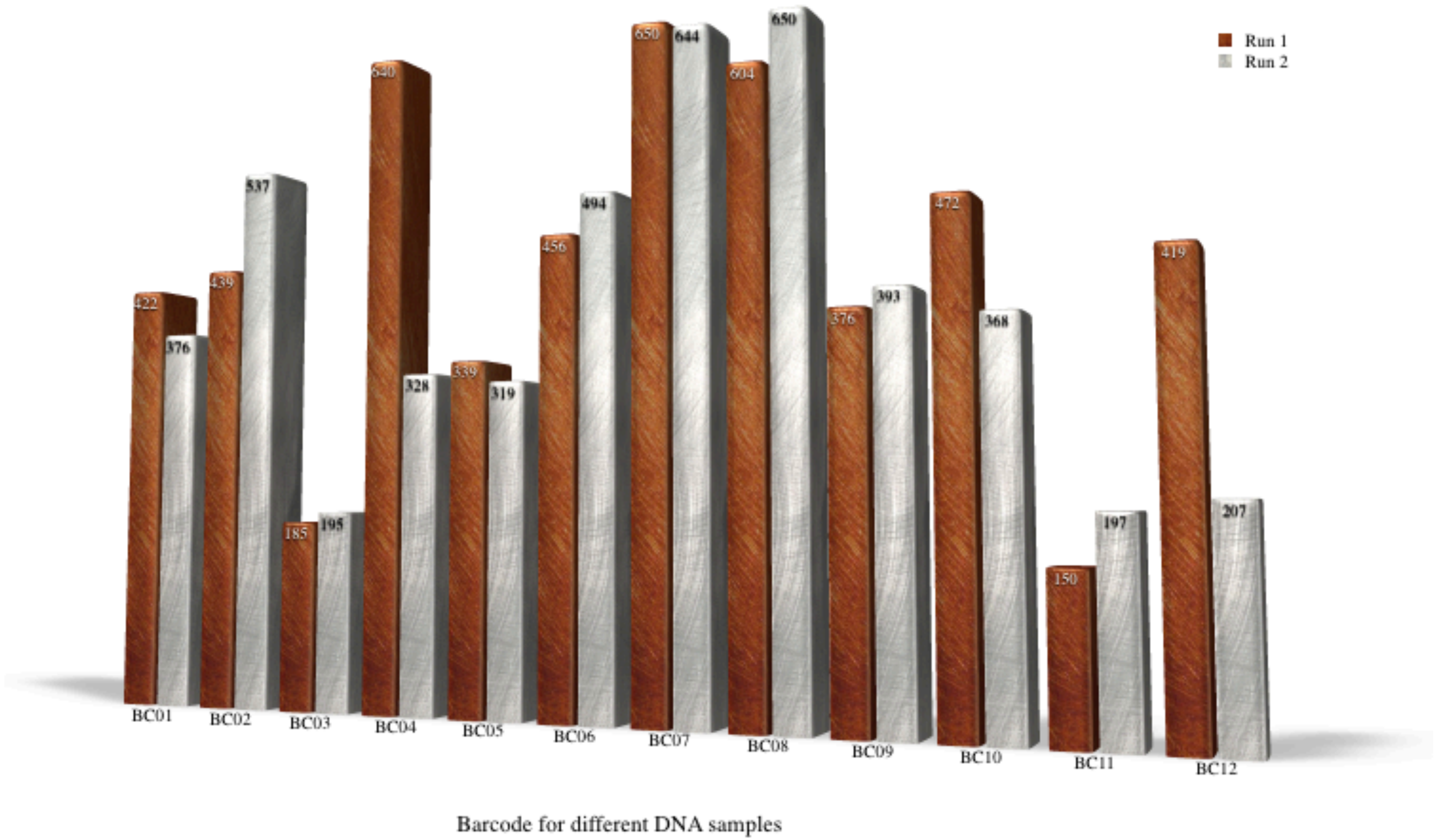
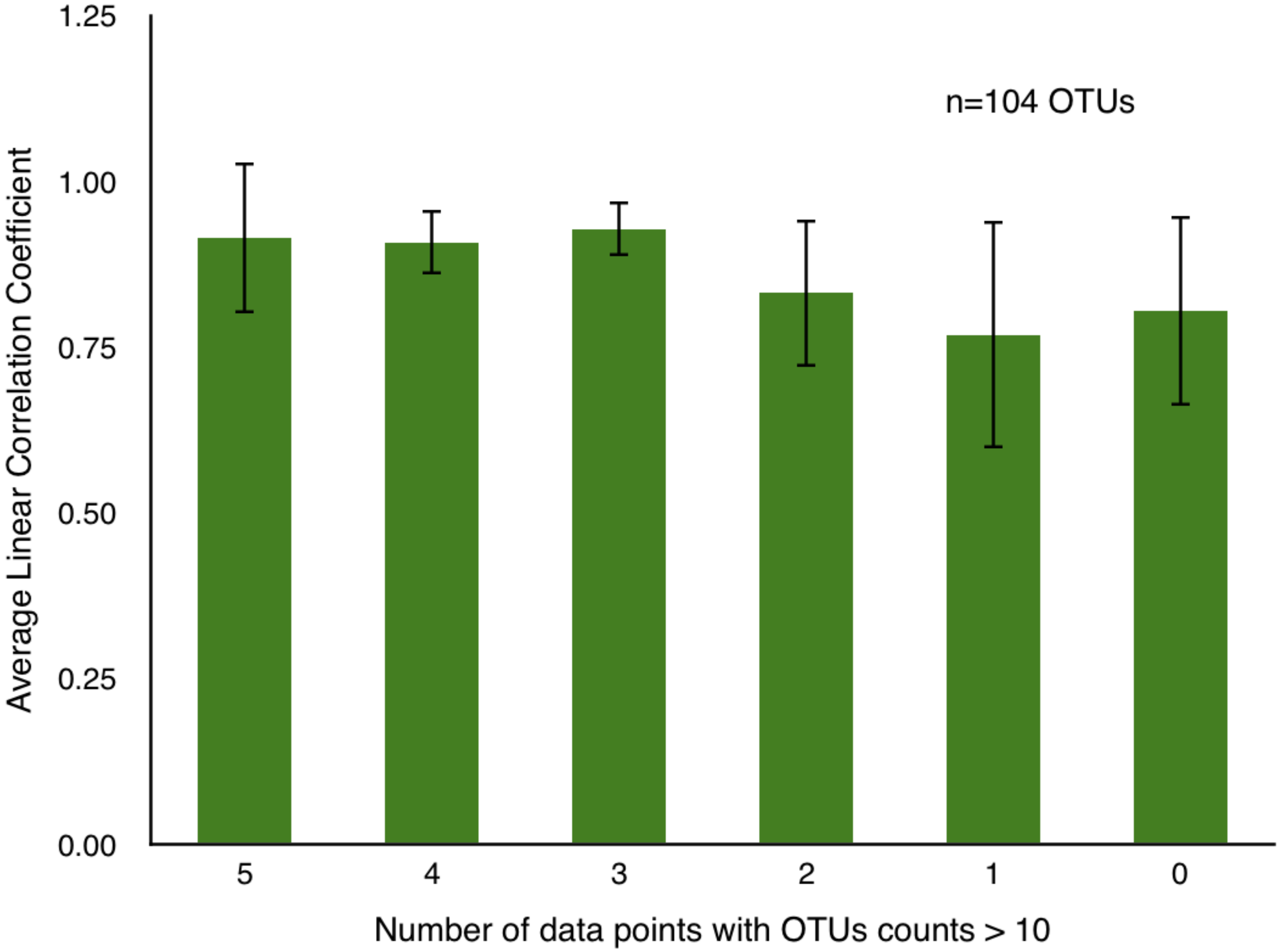
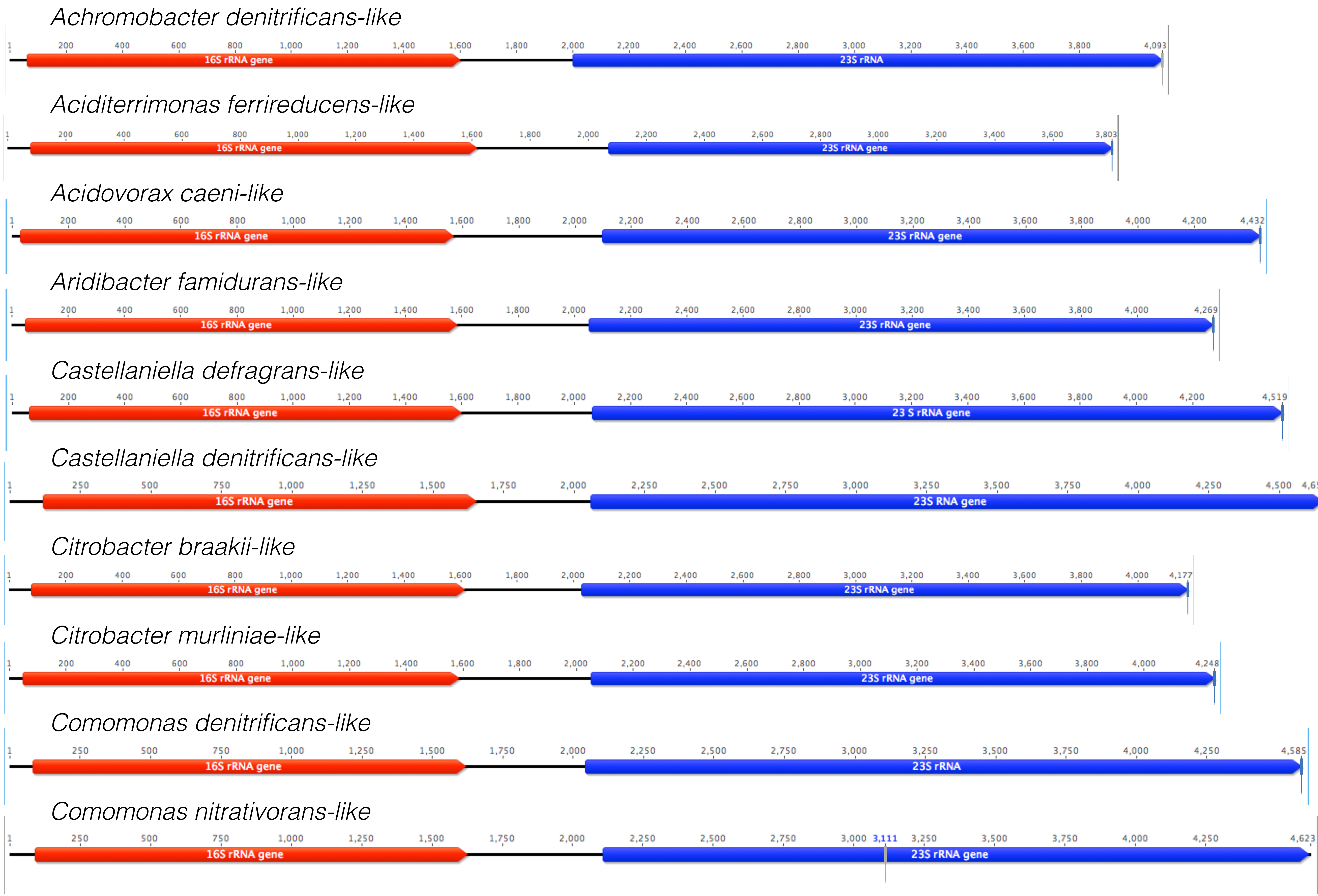

Run 1
Run 2
422
376
439
537
185
195
640
328
339
319
456
494
650
644
604
650
376
393
472
368
150
197
419
207
BC01
BC02
BC03
BC04
BC05
BC06
BC07
BC08
BC09
BC10
BC11
BC12
Barcode for different DNA samples

Figure S7:



Samples are grouped into the number of data points with >10 OTUs. Error bars represent the standard deviation.

Figure S8:



Achromobacter denitrificans-like

Aciditerrimonas ferrireducens-like

Acidovorax caeni-like

Aridibacter famidurans-like

Castellaniella defragrans-like

Castellaniella denitrificans-like

Citrobacter braakii-like

Citrobacter murliniae-like

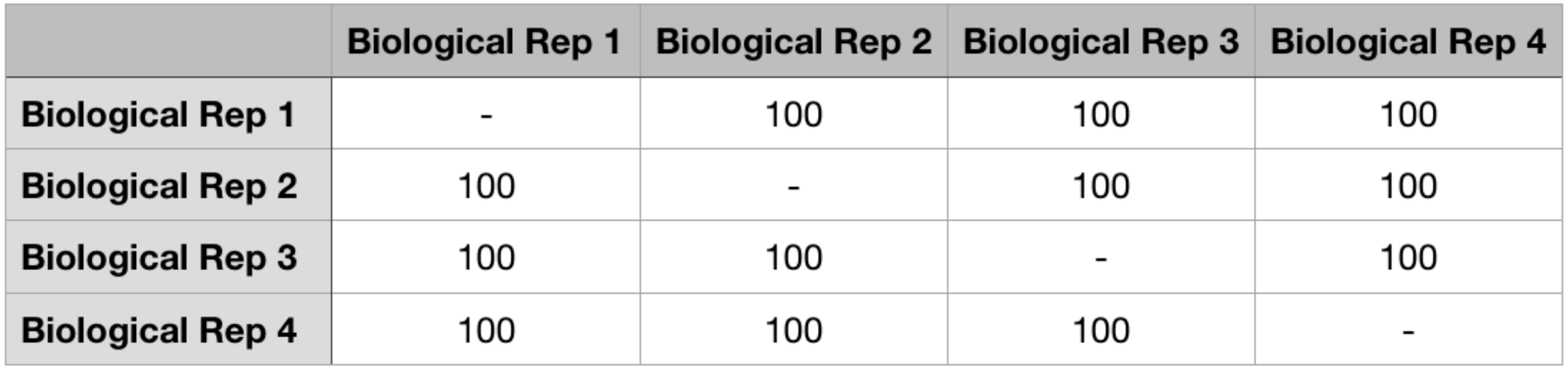
Comomonas denitrificans-like

Comomonas nitrativorans-like

16S rRNA genes (red) and the 23S rRNA genes (blue) indicated.

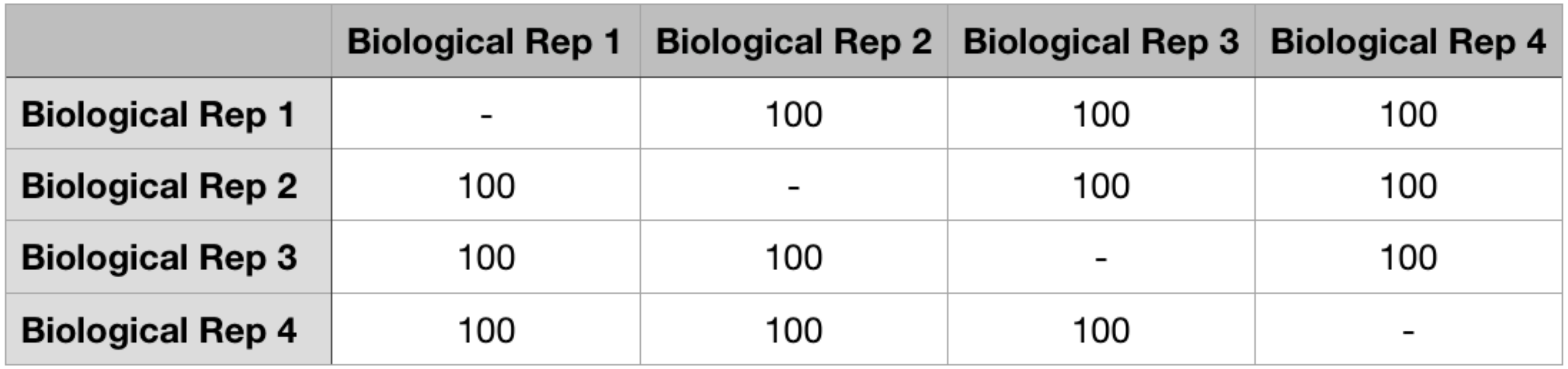
Figure S9:

Acidovorax wautersii



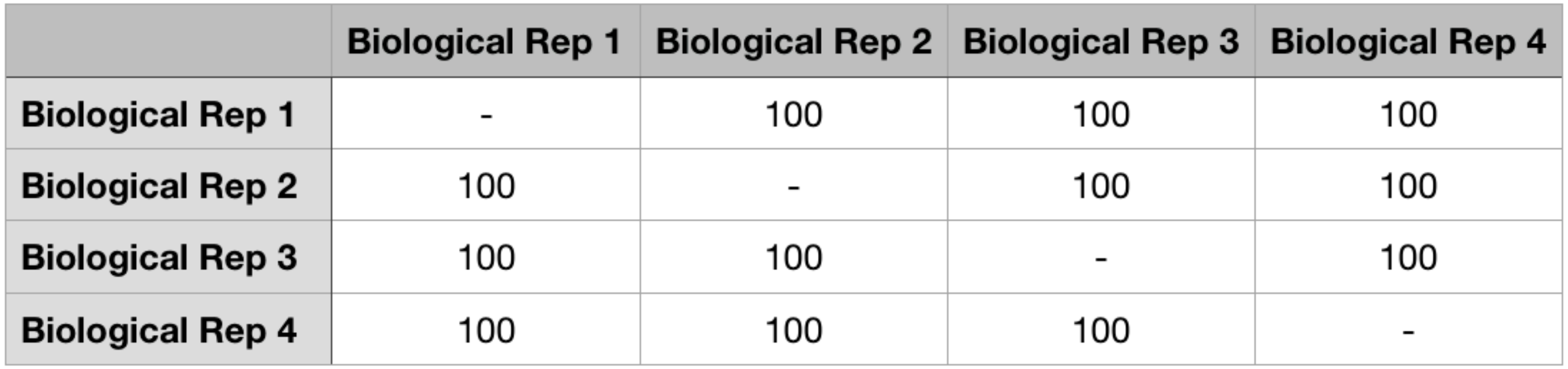
	Biological Rep 1	**Biological Rep 2**	**Biological Rep 3**	**Biological Rep 4**
Biological Rep 1	-	100	100	100
Biological Rep 2	100	-	100	100
Biological Rep 3	100	100	-	100
Biological Rep 4	100	100	100	-

Comomonas nitrativorans



	Biological Rep 1	**Biological Rep 2**	**Biological Rep 3**	**Biological Rep 4**
Biological Rep 1	-	100	100	100
Biological Rep 2	100	-	100	100
Biological Rep 3	100	100	-	100
Biological Rep 4	100	100	100	-

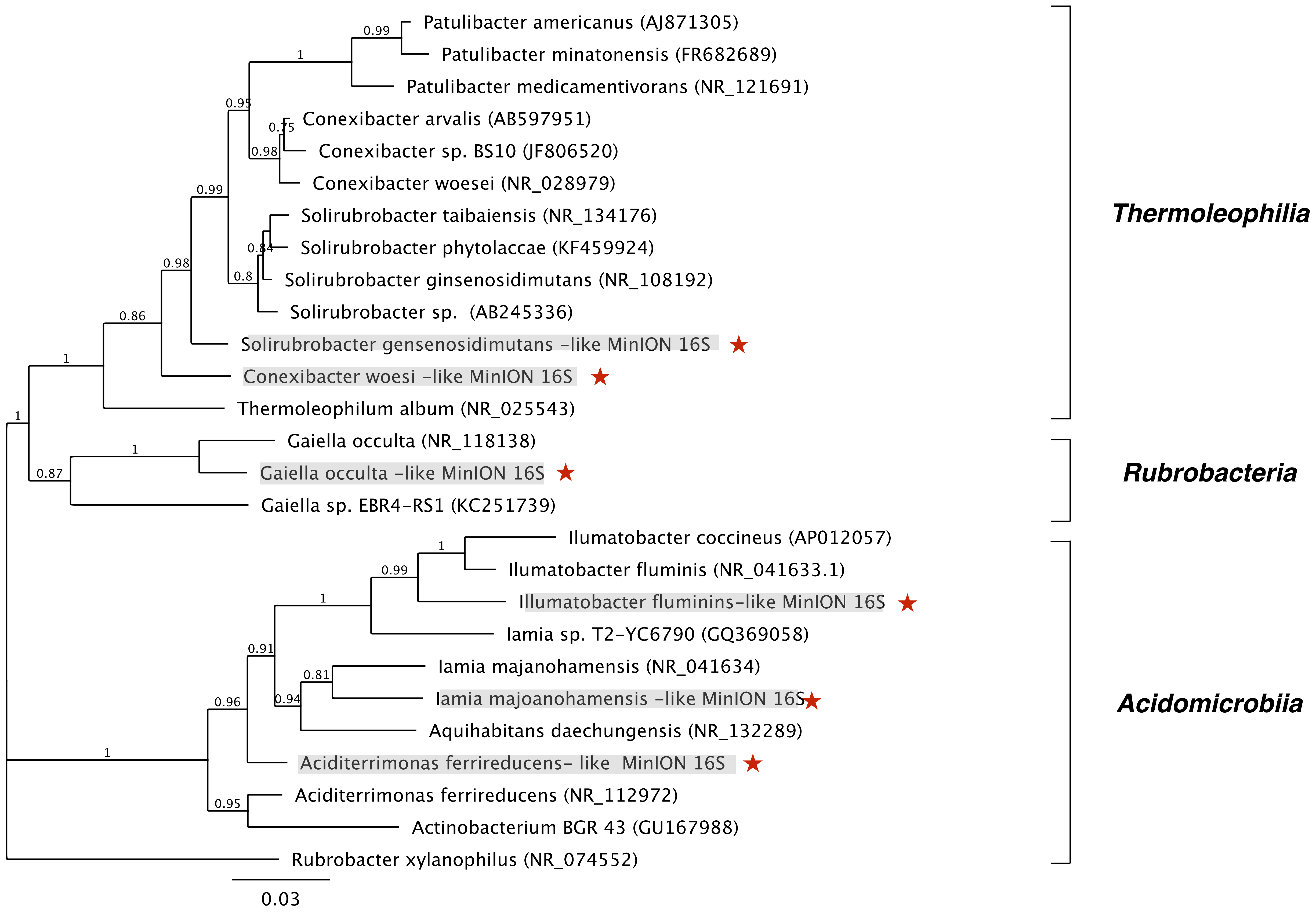
Stenotrophomonas rhizophilia



	Biological Rep 1	**Biological Rep 2**	**Biological Rep 3**	**Biological Rep 4**
Biological Rep 1	-	100	100	100
Biological Rep 2	100	-	100	100
Biological Rep 3	100	100	-	100
Biological Rep 4	100	100	100	-

DNA from each biological replicate was amplified, sequenced, and processed separately to assess errors from consensus building. All 16S rRNA gene consensus sequences from the replicates were found to be identical.

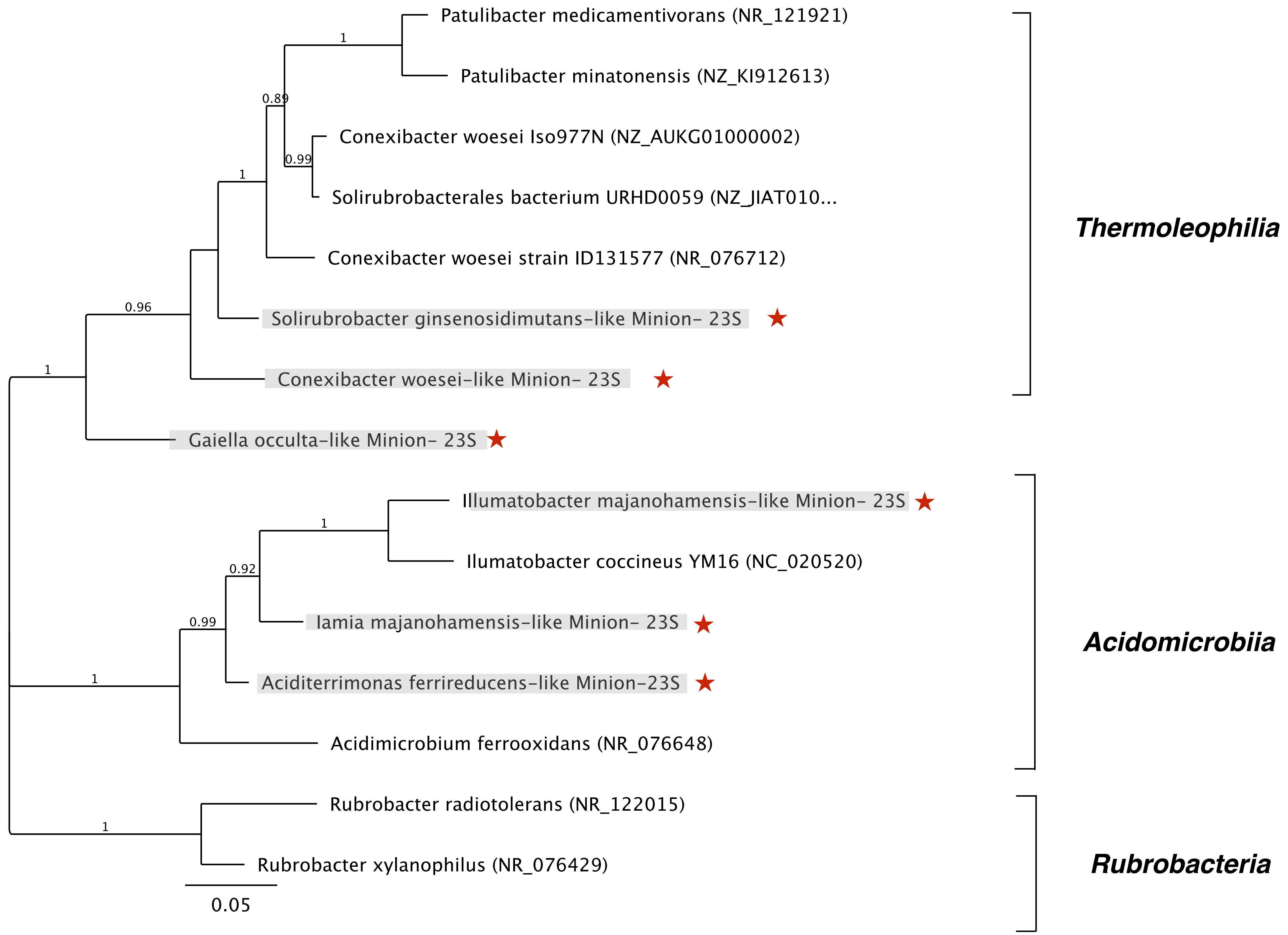
Figure S10:



Tree reconstructions using FastTree for 1252 unambiguously aligned bases.

The MinION sequences are indicated by boxes and stars.

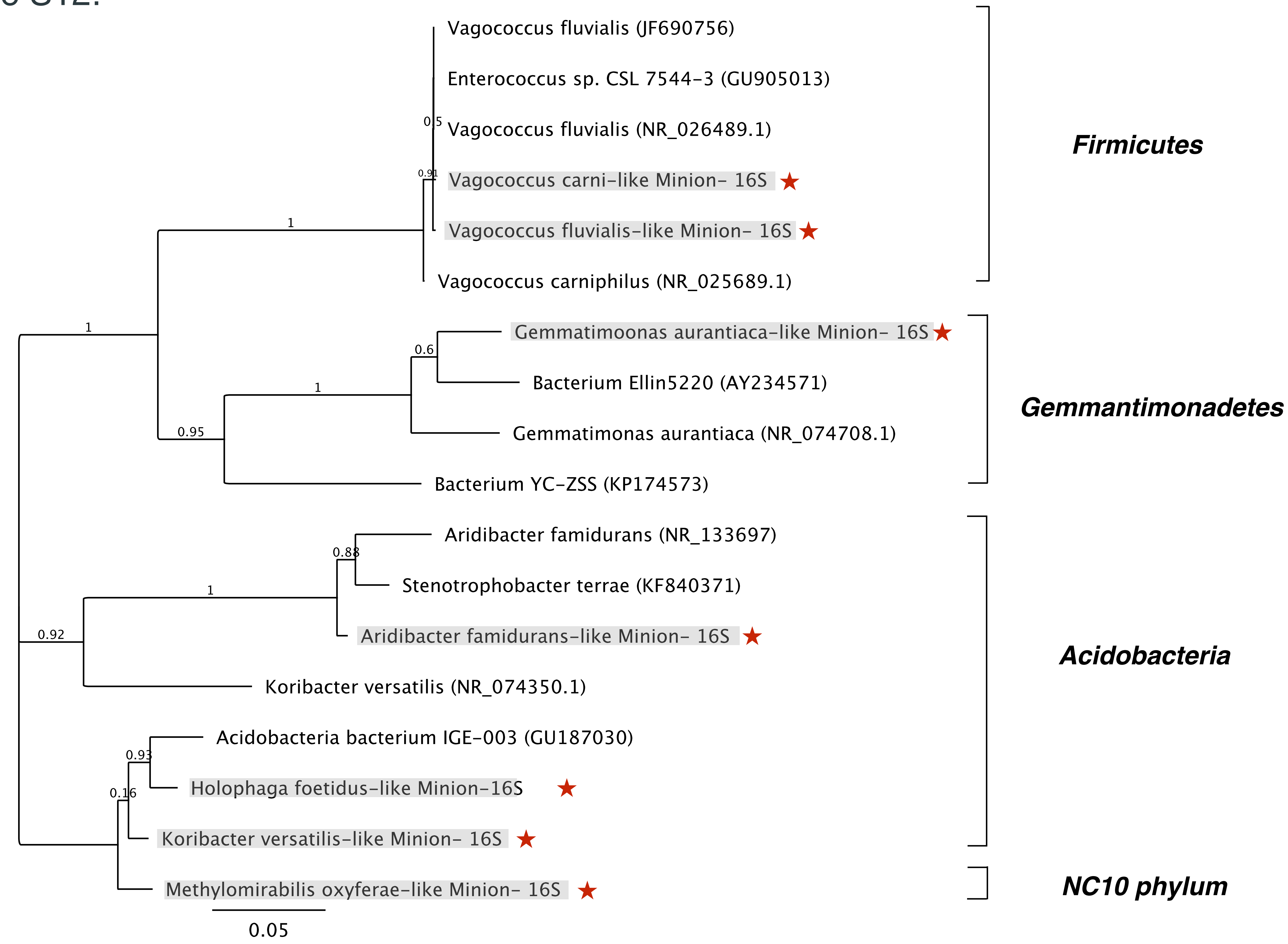
Figure S11:



Tree reconstructions using FastTree for 1553 unambiguously aligned bases.

The MinION sequences are indicated by boxes and stars.

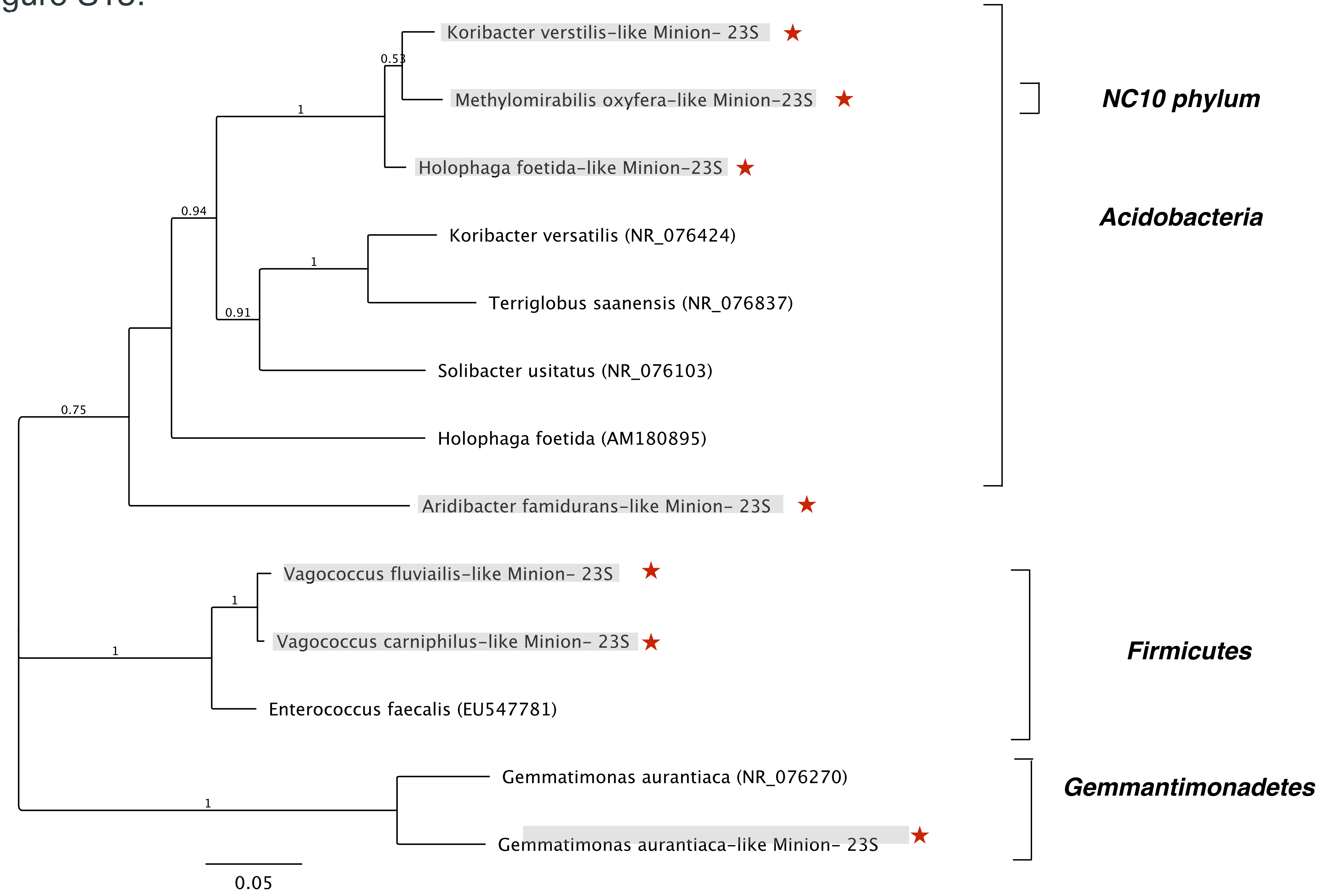
Figure S12:



Tree reconstructions using FastTree for 1181 unambiguously aligned bases.

The MinION sequences are indicated by boxes and stars.

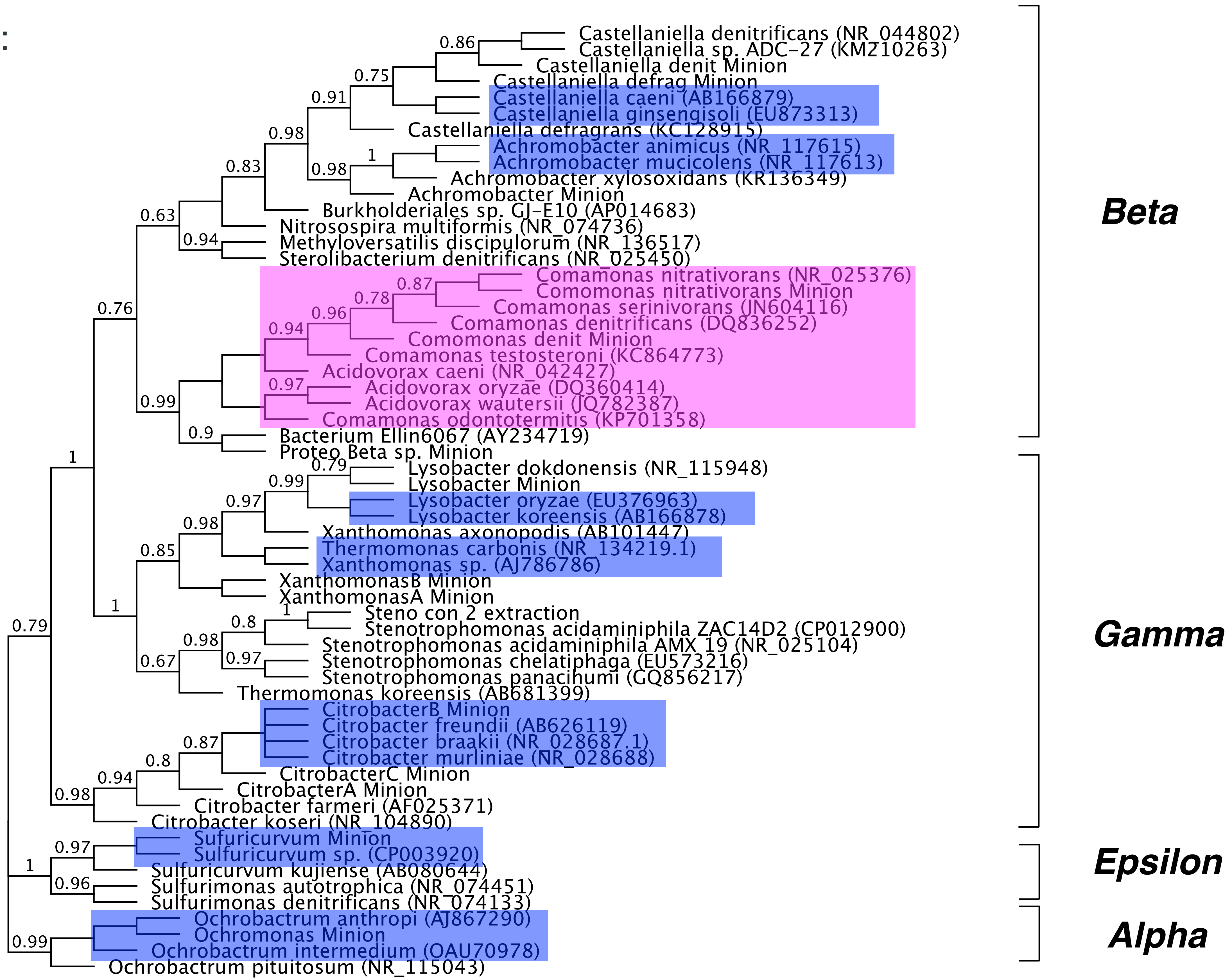
Figure S13:



Tree reconstructions using FastTree for 1518 unambiguously aligned bases.

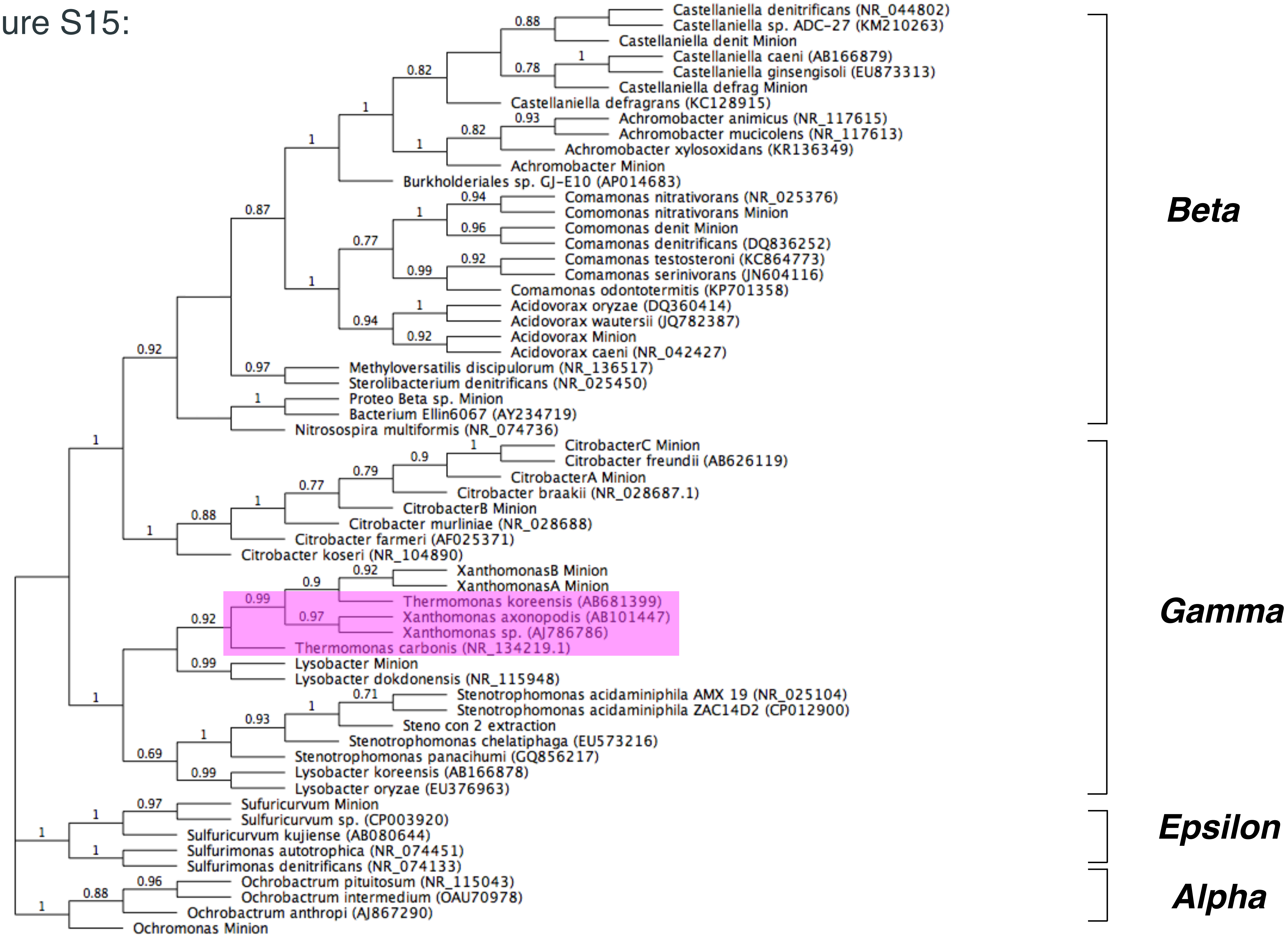
The MinION sequences are indicated by boxes and stars.

Figure S14:



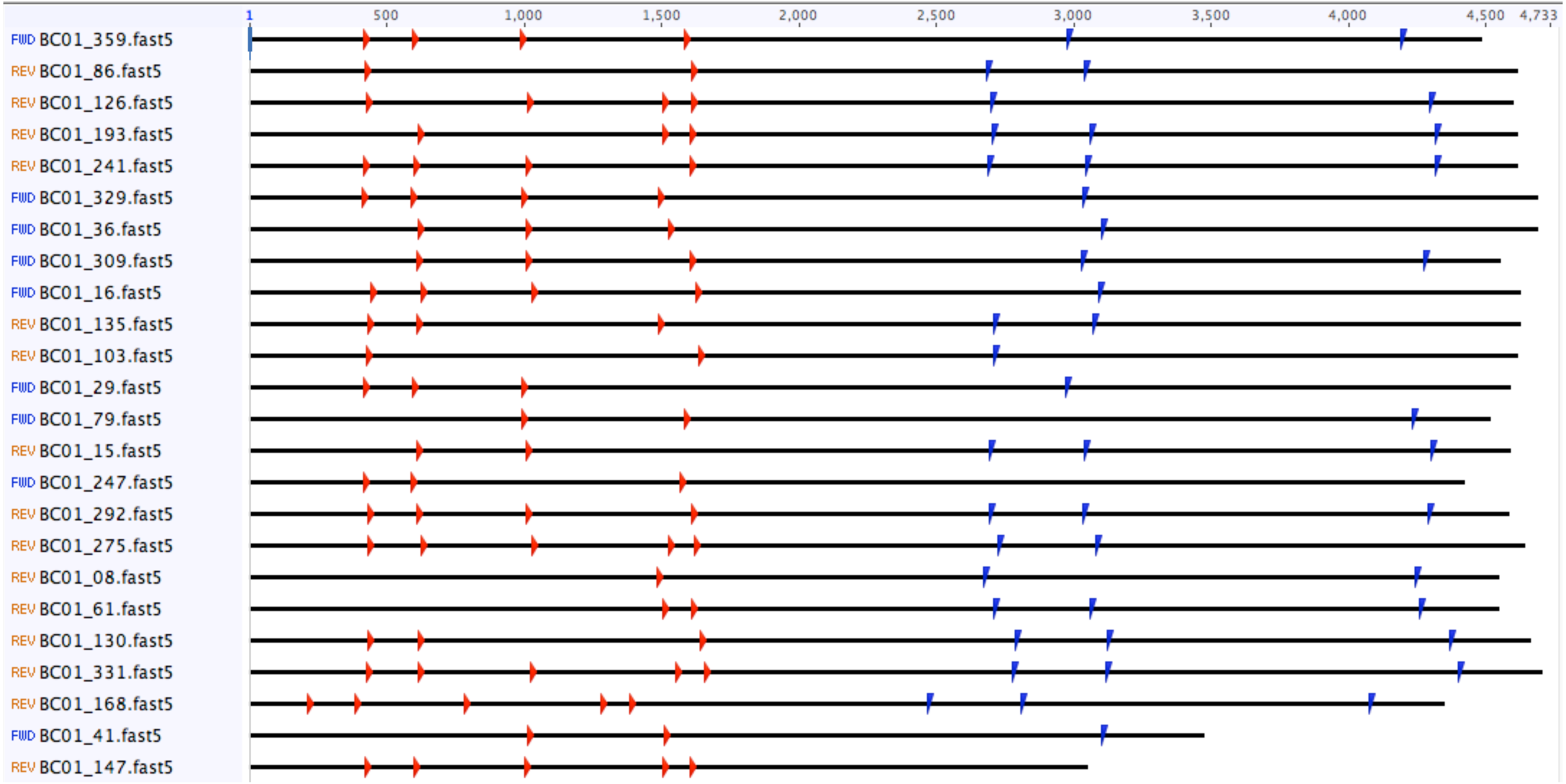
The portion of the tree not supported by bootstrap analysis is highlighted in blue while the portion of the tree not resolving bacterial genera is highlighted in pink.

Figure S15:



The portion of the tree not resolving bacterial genera is highlighted in pink.

Figure S16:



Universal 16S (red) and 23S (blue) rRNA gene priming sets are indicated.

Short sequences (<4000 bp) and those without priming sites are removed during QA/QC.

Table S1:

	2D	1D	Totals
Total Reads	32991	56866	8.99E+04
Total bp	140533277	212346402	3.53E+08
Mean	4238	3698	
Median	4393	4090	
Min	828	13	
Max	8478	169741	
N25	4622	4767	
N50	4433	4383	
N75	4222	3811	

Table S2

Query	Description	Name	Grade	Query coverage	% Pairwise Identity
Sm100	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	100%	100%	100%
Sm99	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	99.5%	100%	98.9%
Sm98	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	98.9%	100%	97.8%
Sm96	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	97.9%	100%	95.8%
Sm94	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	96.8%	100%	93.6%
Sm93	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	96.4%	100%	92.8%
Sm91	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	95.7%	100%	91.4%
Sm90	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	95%	100%	89.9%
Sm89	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	94.5%	100%	88.9%
Sm88	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	94.1%	100%	88.1%
Sm87	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	93.5%	100%	87%
Sm86	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	92.8%	100%	85.5%
SM84	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	92.1%	100%	84.1%
SM83Indel1	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	91.6%	100%	83.1%
SM81Indel2	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	90.7%	100%	81.4%
SM79Indel3	NR_041577.1 Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA gene, complete sequence	9773	90%	100%	79.9%
Cnitrati100	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	100%	100%	100%
Cnitrarti99	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	99.4%	100%	98.9%
Cnitrati98	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	99%	100%	97.9%
Cnitrati96	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	97.8%	100%	95.7%
Cnitrati95	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	97.3%	100%	94.7%
Cnitrati93	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	96.7%	100%	93.3%
cNitrati92	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	95.8%	100%	91.7%
Cnitrati91	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	95.4%	100%	90.9%
Cnitrati90	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	95%	100%	90.1%
Cnitrati89	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	94.4%	100%	88.9%
Cnitriti87	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	93.5%	100%	87%
Cnitrati85	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	92.7%	99.93%	85.4%
Cnitrati81Indel1	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	91.3%	99.94%	82.7%
Cnitrati79Indel2	NR_025376.1 Comamonas nitrativorans strain 23310 16S ribosomal RNA gene, partial sequence	11440	90%	99.94%	80.1%
Cdenit100	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	97.4%	94.87%	99.9%
Cdenit99	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	96.7%	94.87%	98.6%
Cdenit97	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	96%	94.87%	97.2%
Cdenti96	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	95.3%	94.87%	95.7%
Cdenit95	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	94.9%	94.87%	95%
Cdenit94	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	94.3%	94.74%	93.9%
Cdenit92	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	93.6%	94.74%	92.4%
Cdenit90	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	92.4%	94.74%	90.1%
Cdenit89	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	91.9%	94.74%	89%
Cdenit87	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	90.9%	94.74%	87.2%
Cdenit86	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	90.5%	94.74%	86.2%
Cdenit85	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	89.6%	94.74%	84.4%
Cdenit81Indel1	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	87.9%	94.92%	80.9%
Cdenti79Indel2	NR_025080.1 Comamonas denitrificans strain 123 16S ribosomal RNA gene, partial sequence	11567	87.1%	94.89%	79.3%