

# **Analysis of the mouse gut microbiome using full-length 16S rRNA amplicon sequencing**

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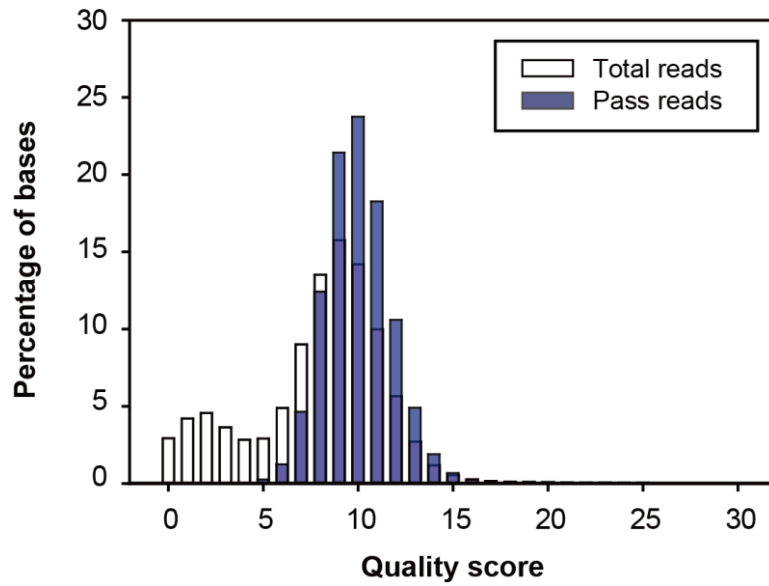
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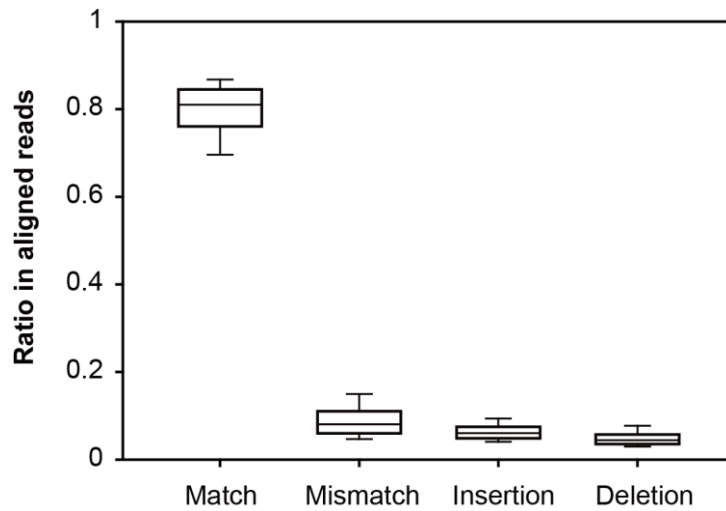
## **Supplementary Data**

Figures S1-S4

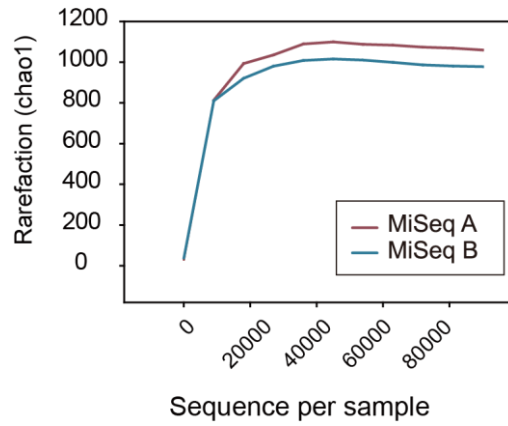
Tables S1-S2



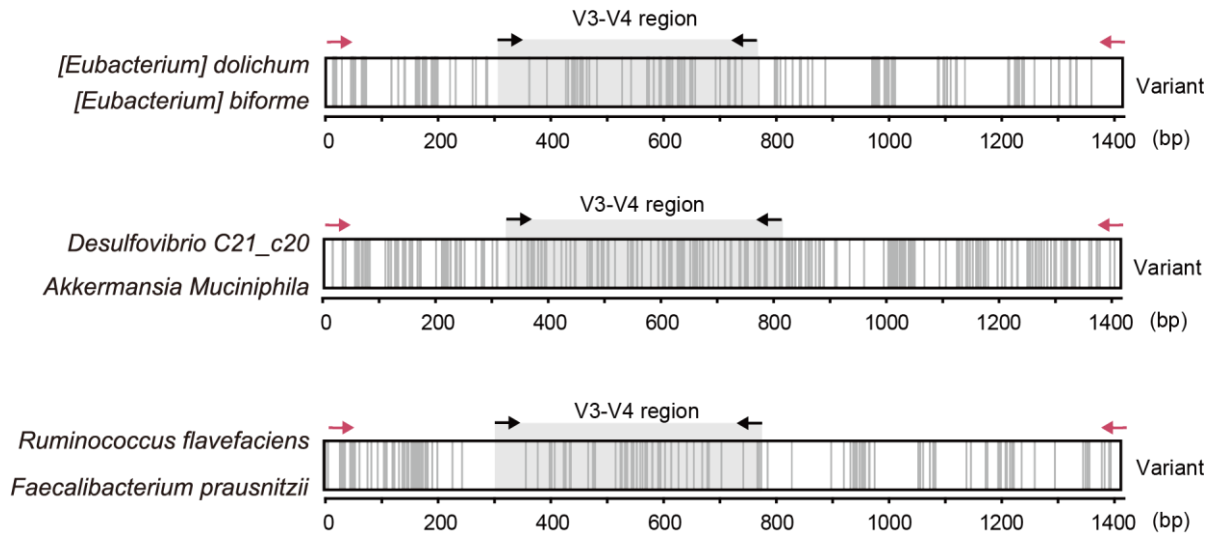
**Supplemental Figure S1. MinION™ quality score distribution.** The distribution of PHRED quality score of MinION™ raw data is plotted on X-axes and percentage of bases on Y-axes for “Total reads” and “Pass 2D reads”.



**Supplemental Figure S2. Comparison of ratios of match, mismatch, insertion and deletion.** The proportion of accuracy was 0.796 in aligned reads. Mismatch, insertion and deletion ratio in aligned reads was 0.090, 0.064 and 0.050, respectively. Box plot show interquartile range with 25<sup>th</sup> and 75<sup>th</sup> percentiles; median is the solid line in each box.



**Supplemental Figure S3. Rarefaction curves of mouse fecal samples based on Illumina MiSeq sequencing.** A total OTUs were generated by 3% distances. Total sample richness estimates were calculated by richness estimators Chao1.



**Supplemental Figure S4. Detected variants between 16S rRNA gene sequences from two phylogenetically related species were represented with vertical line on 16s rDNA sequences.** Variants were defined as nucleotide presents in less than 50% of aligned position frequency. The black and red arrow indicates binding position of primer sets for the amplification of V3–V4 regions and nearly full-regions on 16s rDNA sequences, respectively.

**Supplemental Table S1. Relative abundance of phylotypes detected by MiSeq sequencer.**

Taxonomy	MiSeq A (%)	MiSeq B (%)
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7;	32.808	25.582
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;	20.494	25.279
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Allobaculum;	5.421	10.833
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__acidifaciens;	5.105	2.504
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;	4.244	4.329
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Sutterella;	3.627	4.076
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__YS2;	3.531	0.774
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;	3.195	5.372
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;	2.257	2.109
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus];s__gnavus;	2.064	1.149
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;	1.962	1.819
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira;	1.864	1.554
k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter;	1.510	1.282
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides;	1.429	0.510
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;	1.383	1.257

Taxonomy	MiSeq	MiSeq
	A (%)	B (%)
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;	1.125	1.272
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;s__flavefaciens;	1.077	0.149
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;	0.901	1.195
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coproccoccus;	0.738	0.714
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;	0.717	0.123
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae;g__Anaeroplasmata;	0.673	0.103
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;	0.463	0.745
Unassigned;	0.416	0.268
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerostipes;	0.413	0.106
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Dorea;	0.337	0.512
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Coproccoccus;	0.303	0.005
k__Bacteria;p__TM7;c__TM7-3;o__CW040;f__F16;	0.276	0.220
k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Akkermansia;s__muciniphila;	0.273	4.733
k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39;	0.252	0.162
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Dehalobacteriaceae;g__Dehalobacterium;	0.238	0.109
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__	0.154	0.002

<b>Taxonomy</b>	<b>MiSeq A (%)</b>	<b>MiSeq B (%)</b>
_rc4-4;		
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__RF32;	0.140	0.037
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium;	0.104	0.250
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__reuteri;	0.086	0.085
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;	0.061	0.020
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Adlercreutzia;	0.044	0.210
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio;	0.043	0.322
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;	0.035	0.004
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;	0.034	0.031
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;	0.033	0.015
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;	0.031	0.007
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];	0.025	0.025
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium;s__perfringens;	0.024	0.004
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium;andidatus Arthromitus;	0.022	0.029
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;	0.018	0.013
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;	0.017	0.004
k__Bacteria;p__Firmicutes;	0.007	0.003
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;	0.006	0.011



Taxonomy	MiSeq	MiSeq
	A (%)	B (%)
acteriaceae;g__Bifidobacterium;s__pseudolongum;		
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;	0.003	0.007
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae;g__Anaerofustis;	0.003	0.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Blautia;	0.003	0.000
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;	0.003	0.003
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__ovatus;	0.002	0.000
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta;	0.002	0.007
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;g__Christensenella;	0.002	0.003
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__mitochondria;	0.002	0.025
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;	0.002	0.009
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Actinomycetospora;	0.000	0.002
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;	0.000	0.002
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus;	0.000	0.002
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;	0.000	0.002
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;	0.000	0.002

<b>Taxonomy</b>	<b>MiSeq</b>	<b>MiSeq</b>
	<b>A (%)</b>	<b>B (%)</b>
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;	0.000	0.010
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter;	0.000	0.004
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter;s__rhizosphaerae	0.000	0.002
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;	0.000	0.002

**Supplemental Table S2. Relative abundance of phylotypes detected by MinION™ sequencer.**

Taxonomy	MinION A (%)	MinION B (%)
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7;	57.640	56.168
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;	10.641	11.355
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Allobaculum;	7.750	8.692
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;	3.166	2.944
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__acidifaciens;	2.172	2.383
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Sutterella;	1.970	2.103
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;	1.491	1.636
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;	1.473	1.822
k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39;	1.178	1.028
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__YS2;	1.141	1.308
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus];s__gnavus;	1.049	0.841
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira;	0.773	0.607
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;s__flavefaciens;	0.736	0.654
k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter;	0.681	0.561
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;	0.681	0.374

Taxonomy	MinION	MinION
	A (%)	B (%)
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Dorea;	0.663	0.794
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;	0.663	0.607
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides;	0.626	0.561
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;	0.571	0.841
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;	0.552	0.701
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;	0.534	0.701
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__RF32;	0.479	0.607
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;	0.460	0.280
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus;	0.368	0.327
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__pseudolongum;	0.350	0.280
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;	0.313	0.421
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerostipes;	0.221	0.234
k__Bacteria;p__TM7;c__TM7-3;o__CW040;f__F16;	0.203	0.280
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;	0.184	0.000
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae;g__Anaeroplasma;	0.147	0.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Dehalobacteriaceae;g__Dehalobacterium;	0.147	0.093

Taxonomy	MinION	MinION
	A (%)	B (%)
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__rc4-4;	0.129	0.140
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Coprobacillus;	0.110	0.187
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Blautia;	0.092	0.093
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;	0.074	0.000
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella;s__copri;	0.055	0.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus];	0.055	0.000
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__animalis;	0.055	0.000
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides;s__distasonis;	0.055	0.000
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio;s__C21_c20;	0.055	0.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Faecalibacterium;s__prausnitzii;	0.037	0.000
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella;	0.037	0.000
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__[Eubacterium];s__dolichum;	0.037	0.000
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__reuteri;	0.037	0.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium;	0.037	0.187

<b>Taxonomy</b>	<b>MinION A (%)</b>	<b>MinION B (%)</b>
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__ovatus;	0.037	0.000
k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Akkermansia;s__muciniphila;	0.037	0.093
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__[Eubacterium];s__biforme;	0.037	0.000
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];	0.000	0.093