

Assessing the Differences in 16S rRNA Gene Sequencing Due to High Fidelity DNA Polymerase

Marc A Sze and Patrick D Schloss

Supplemental

Table S1: Wilcoxon Rank Sum Results of High versus Low GC groups based on the median percent of the V4 hypervariable region or the whole genome

Number of Cycles	Polymerase	Rank Sum Statistic	P-value	BH	Test Type
30x	PL	404.0	9.7e-05	2.4e-03	V4 Region
35x	PL	56.0	5.8e-03	5.4e-02	V4 Region
20x	PHU	59.0	8.5e-03	5.4e-02	V4 Region
30x	Q5	532.0	9.5e-03	5.4e-02	V4 Region
25x	PL	61.0	1.1e-02	5.4e-02	V4 Region
30x	K	546.0	1.5e-02	5.4e-02	V4 Region
25x	PHU	64.0	1.5e-02	5.4e-02	V4 Region
20x	PL	69.0	2.6e-02	8.1e-02	V4 Region
30x	PHU	74.0	4.3e-02	1.1e-01	V4 Region
35x	PHU	77.0	5.6e-02	1.3e-01	V4 Region
25x	ACC	82.5	9.0e-02	1.9e-01	V4 Region
25x	K	85.0	1.1e-01	1.9e-01	V4 Region
35x	ACC	88.0	1.4e-01	2.3e-01	V4 Region
20x	ACC	90.0	1.6e-01	2.5e-01	V4 Region
25x	Q5	96.0	2.4e-01	3.3e-01	V4 Region
20x	Q5	98.0	2.7e-01	3.6e-01	V4 Region
35x	Q5	99.0	2.9e-01	3.6e-01	V4 Region
35x	K	102.5	3.5e-01	4.1e-01	V4 Region
20x	K	108.0	4.6e-01	5.3e-01	V4 Region
30x	ACC	139.0	7.0e-01	7.6e-01	V4 Region
30x	Q5	1340.0	4.1e-08	1.0e-06	Whole Genome
30x	K	1354.5	9.8e-08	1.2e-06	Whole Genome
20x	PL	249.0	1.5e-07	1.2e-06	Whole Genome
25x	PHU	237.0	6.9e-06	2.9e-05	Whole Genome
25x	PL	237.0	6.9e-06	2.9e-05	Whole Genome
25x	Q5	227.0	7.1e-05	2.5e-04	Whole Genome

Number of Cycles	Polymerase	Rank Sum Statistic	P-value	BH	Test Type
20x	Q5	224.0	1.3e-04	4.0e-04	Whole Genome
20x	PHU	220.0	2.7e-04	7.6e-04	Whole Genome
25x	ACC	223.5	3.4e-04	7.8e-04	Whole Genome
35x	K	223.5	3.4e-04	7.8e-04	Whole Genome
30x	ACC	217.0	4.7e-04	9.7e-04	Whole Genome
25x	K	210.0	2.1e-03	4.1e-03	Whole Genome
30x	PHU	206.0	2.6e-03	4.4e-03	Whole Genome
20x	K	201.0	6.3e-03	9.8e-03	Whole Genome
20x	ACC	194.0	1.4e-02	2.0e-02	Whole Genome
35x	PHU	186.0	2.9e-02	4.0e-02	Whole Genome
30x	PL	961.0	1.2e-01	1.5e-01	Whole Genome
35x	Q5	168.0	1.4e-01	1.6e-01	Whole Genome
35x	ACC	152.0	3.8e-01	4.1e-01	Whole Genome
35x	PL	115.0	6.4e-01	6.7e-01	Whole Genome

Table S2: Kruskal-Wallis results of differences in error rate, sequences with an error, and chimera prevalence between polymerases in mock samples

Rank Sum Statistic	DF	P-value	BH	Cycle	Test Type
7.85	2	2.0e-02	2.0e-02	20x	Error Rate
12.95	4	1.2e-02	1.5e-02	25x	Error Rate
28.31	4	1.1e-05	4.3e-05	30x	Error Rate
15.00	4	4.7e-03	9.4e-03	35x	Error Rate
7.85	2	2.0e-02	2.0e-02	20x	Sequences with Error
15.98	4	3.0e-03	5.2e-03	25x	Sequences with Error
29.22	4	7.0e-06	2.8e-05	30x	Sequences with Error
15.44	4	3.9e-03	5.2e-03	35x	Sequences with Error
8.91	2	1.2e-02	1.2e-02	20x	Chimera Prevalence
15.54	4	3.7e-03	4.9e-03	25x	Chimera Prevalence
21.41	4	2.6e-04	1.1e-03	30x	Chimera Prevalence
16.28	4	2.7e-03	4.9e-03	35x	Chimera Prevalence

Table S3: Dunn's Post-Hoc results of differences in error rate, sequences with an error, and chimera prevalence between polymerases in mock samples

Chi Squared	Z Statistic	P Adjusted	Comparison	Cycle	Test Type
7.85	2.77	2.8e-03	PHU - PL	20x	Error Rate
7.85	-1.64	5.0e-02	PL - Q5	20x	Error Rate
12.95	3.26	5.6e-04	K - Q5	25x	Error Rate
12.95	2.68	3.7e-03	K - PHU	25x	Error Rate
12.95	2.01	2.2e-02	ACC - Q5	25x	Error Rate
12.95	1.88	3.0e-02	PL - Q5	25x	Error Rate
28.31	-4.46	4.1e-06	ACC - K	30x	Error Rate
28.31	4.32	7.7e-06	K - PL	30x	Error Rate
28.31	-2.69	3.6e-03	ACC - Q5	30x	Error Rate
28.31	2.65	4.0e-03	K - PHU	30x	Error Rate
28.31	2.37	8.8e-03	K - Q5	30x	Error Rate
28.31	-2.00	2.3e-02	PL - Q5	30x	Error Rate
15.00	3.14	8.6e-04	K - PHU	35x	Error Rate
15.00	-3.05	1.2e-03	PHU - PL	35x	Error Rate
15.00	-2.33	1.0e-02	ACC - K	35x	Error Rate
15.00	-2.05	2.0e-02	ACC - PL	35x	Error Rate
15.00	-1.79	3.7e-02	PHU - Q5	35x	Error Rate
15.00	1.68	4.7e-02	K - Q5	35x	Error Rate
7.85	2.77	2.8e-03	PHU - PL	20x	Sequences with Error
7.85	-1.64	5.0e-02	PL - Q5	20x	Sequences with Error
15.98	3.37	3.7e-04	K - Q5	25x	Sequences with Error
15.98	2.79	2.6e-03	K - PHU	25x	Sequences with Error
15.98	2.76	2.9e-03	ACC - Q5	25x	Sequences with Error
15.98	2.14	1.6e-02	ACC - PHU	25x	Sequences with Error
15.98	1.86	3.1e-02	K - PL	25x	Sequences with Error
29.22	4.17	1.5e-05	K - Q5	30x	Sequences with Error

Chi Squared	Z Statistic	P Adjusted	Comparison	Cycle	Test Type
29.22	3.89	5.0e-05	K - PHU	30x	Sequences with Error
29.22	3.57	1.8e-04	PL - Q5	30x	Sequences with Error
29.22	-3.41	3.2e-04	PHU - PL	30x	Sequences with Error
29.22	-1.81	3.5e-02	ACC - K	30x	Sequences with Error
29.22	1.76	3.9e-02	ACC - PHU	30x	Sequences with Error
15.44	-3.71	1.0e-04	PHU - PL	35x	Sequences with Error
15.44	2.38	8.6e-03	PL - Q5	35x	Sequences with Error
15.44	2.38	8.7e-03	K - PHU	35x	Sequences with Error
15.44	1.85	3.2e-02	ACC - PHU	35x	Sequences with Error
15.44	-1.85	3.2e-02	ACC - PL	35x	Sequences with Error
8.91	2.98	1.4e-03	PHU - PL	20x	Chimera Prevalence
15.54	3.37	3.7e-04	K - PHU	25x	Chimera Prevalence
15.54	-2.76	2.9e-03	PHU - PL	25x	Chimera Prevalence
15.54	-2.68	3.7e-03	ACC - K	25x	Chimera Prevalence
15.54	-2.01	2.2e-02	ACC - PL	25x	Chimera Prevalence
15.54	1.98	2.4e-02	K - Q5	25x	Chimera Prevalence
21.41	-4.23	1.2e-05	ACC - PL	30x	Chimera Prevalence
21.41	2.89	1.9e-03	PL - Q5	30x	Chimera Prevalence
21.41	-2.84	2.2e-03	ACC - K	30x	Chimera Prevalence
21.41	-2.59	4.8e-03	PHU - PL	30x	Chimera Prevalence
21.41	-2.05	2.0e-02	ACC - Q5	30x	Chimera Prevalence
21.41	-1.73	4.2e-02	K - PL	30x	Chimera Prevalence
16.28	-3.71	1.0e-04	ACC - PL	35x	Chimera Prevalence
16.28	-2.65	4.0e-03	PHU - PL	35x	Chimera Prevalence
16.28	-2.38	8.7e-03	ACC - K	35x	Chimera Prevalence
16.28	-2.12	1.7e-02	ACC - Q5	35x	Chimera Prevalence

Table S4: ANOVA results of differences in number of OTUs between polymerases in fecal and mock samples

DF	Sum Squares	Mean Squares	F value	P-value	BH	Cycle	Sample Type
4	3.66	0.91	1.25	3.5e-01	3.5e-01	20x	Fecal Sample
4	6.90	1.73	2.84	6.1e-02	1.2e-01	25x	Fecal Sample
3	4.67	1.56	2.55	1.0e-01	1.4e-01	30x	Fecal Sample
4	13.01	3.25	16.35	2.4e-05	9.7e-05	35x	Fecal Sample
2	364.56	182.28	15.82	1.7e-03	2.2e-03	20x	Mock Sample
4	3478.53	869.63	75.15	2.7e-09	1.1e-08	25x	Mock Sample
4	2206.58	551.64	10.28	1.8e-05	3.6e-05	30x	Mock Sample
4	1971.92	492.98	2.31	1.0e-01	1.0e-01	35x	Mock Sample

Table S5: Tukey post-hoc results of differences in number of OTUs between polymerases in fecal and mock samples

Difference	Lower	Upper	P Adjusted	Comparison	Cycle	Sample Type
25.00	-17.32	67.32	0.40	PL-ACC	35x	Fecal Sample
24.25	-18.07	66.57	0.43	PL-K	35x	Fecal Sample
20.50	-21.82	62.82	0.58	Q5-ACC	35x	Fecal Sample
19.75	-22.57	62.07	0.61	Q5-K	35x	Fecal Sample
13.50	-28.82	55.82	0.86	PHU-ACC	35x	Fecal Sample
12.75	-29.57	55.07	0.88	PHU-K	35x	Fecal Sample
11.50	-30.82	53.82	0.91	PL-PHU	35x	Fecal Sample
7.00	-35.32	49.32	0.98	Q5-PHU	35x	Fecal Sample
-4.50	-46.82	37.82	1.00	Q5-PL	35x	Fecal Sample
0.75	-41.57	43.07	1.00	K-ACC	35x	Fecal Sample
-13.50	-20.36	-6.64	1.3e-03	PL-PHU	20x	Mock Sample
-38.50	-46.60	-30.40	5.1e-09	Q5-K	25x	Mock Sample
-37.50	-45.60	-29.40	7.3e-09	PHU-K	25x	Mock Sample
36.50	28.40	44.60	1.1e-08	K-ACC	25x	Mock Sample
-35.50	-43.60	-27.40	1.5e-08	PL-K	25x	Mock Sample
22.03	9.31	34.74	1.8e-04	K-ACC	30x	Mock Sample
-15.48	-25.20	-5.75	5.7e-04	Q5-K	30x	Mock Sample
-18.78	-31.49	-6.06	1.5e-03	PHU-K	30x	Mock Sample
15.55	3.03	28.07	9.0e-03	PL-ACC	30x	Mock Sample

Table S6: Kruskal-Wallis results of significant differences in OTU in mock community samples

Cycles	OTU	Lowest Taxa ID	Statistic	P-value	BH	Sample Type
25x	Otu0033	Clostridiales	18.00000	1.2e-03	1.6e-02	mock
25x	Otu0039	Phascolarctobacterium	18.00000	1.2e-03	1.6e-02	mock
25x	Otu0024	Dorea	17.88235	1.3e-03	1.6e-02	mock
25x	Otu0026	Roseburia	17.88235	1.3e-03	1.6e-02	mock
25x	Otu0031	Erysipelotrichaceae	17.88235	1.3e-03	1.6e-02	mock
25x	Otu0068	Blautia	17.88235	1.3e-03	1.6e-02	mock
25x	Otu0006	Roseburia	17.84348	1.3e-03	1.6e-02	mock
25x	Otu0009	Ruminococcaceae	17.84348	1.3e-03	1.6e-02	mock
25x	Otu0012	Blautia	17.84348	1.3e-03	1.6e-02	mock
25x	Otu0030	Enterococcus	17.84348	1.3e-03	1.6e-02	mock
30x	Otu0185	Streptococcus	30.54780	3.8e-06	1.1e-03	mock
30x	Otu0020	Pseudomonas	28.11429	1.2e-05	1.7e-03	mock
30x	Otu0023	Bacteroides	26.88809	2.1e-05	2.0e-03	mock
30x	Otu0007	Escherichia/Shigella	23.06969	1.2e-04	8.6e-03	mock
30x	Otu0011	Bacillales	21.61181	2.4e-04	1.3e-02	mock
30x	Otu0017	Lactobacillus	21.11598	3.0e-04	1.4e-02	mock
30x	Otu0004	Staphylococcus	20.10917	4.8e-04	1.6e-02	mock
30x	Otu0001	Salmonella	19.96365	5.1e-04	1.6e-02	mock
30x	Otu0162	Bacillales	19.74476	5.6e-04	1.6e-02	mock
30x	Otu0029	Bacteroides	19.02599	7.8e-04	1.6e-02	mock
30x	Otu0030	Enterococcus	18.17396	1.1e-03	1.6e-02	mock
30x	Otu0056	Bacilli	18.09753	1.2e-03	1.6e-02	mock
30x	Otu0083	Alistipes	16.91062	2.0e-03	2.3e-02	mock
35x	Otu0031	Erysipelotrichaceae	19.95000	5.1e-04	1.6e-02	mock

Table S7: Significant Dunns post-hoc results of differences between polymerases for specific taxa

Chi Sq	Z	P-value	BH	Comparison	Cycles	OTU	Taxa	Part of Mock
2.0e+01	-4.2e+00	1.1e-05	1.1e-04	ACC - PL	30x	Otu0001	Salmonella	yes
2.3e+01	4.0e+00	3.8e-05	3.8e-04	PL - Q5	30x	Otu0007	Escherichia/Shigella	yes
2.3e+01	-3.7e+00	9.9e-05	5.0e-04	K - PL	30x	Otu0007	Escherichia/Shigella	yes
1.8e+01	-3.5e+00	2.5e-04	6.3e-04	ACC - K	25x	Otu0030	Enterococcus	yes
2.3e+01	-3.5e+00	2.0e-04	6.8e-04	ACC - PL	30x	Otu0007	Escherichia/Shigella	yes
1.8e+01	3.5e+00	2.5e-04	8.4e-04	K - PHU	25x	Otu0030	Enterococcus	yes
1.8e+01	3.7e+00	1.1e-04	1.1e-03	K - Q5	30x	Otu0030	Enterococcus	yes
1.8e+01	3.5e+00	2.5e-04	1.3e-03	K - PL	25x	Otu0030	Enterococcus	yes
2.0e+01	3.6e+00	1.4e-04	1.4e-03	ACC - PL	30x	Otu0004	Staphylococcus	yes
2.0e+01	3.4e+00	3.8e-04	1.9e-03	K - PL	30x	Otu0004	Staphylococcus	yes
1.8e+01	3.5e+00	2.5e-04	2.5e-03	K - Q5	25x	Otu0030	Enterococcus	yes
1.8e+01	3.2e+00	6.6e-04	3.3e-03	K - PL	30x	Otu0030	Enterococcus	yes
1.8e+01	-2.8e+00	2.3e-03	5.9e-03	ACC - K	30x	Otu0030	Enterococcus	yes
1.8e+01	2.8e+00	2.3e-03	7.8e-03	K - PHU	30x	Otu0030	Enterococcus	yes
2.0e+01	2.7e+00	3.9e-03	1.3e-02	ACC - PHU	30x	Otu0004	Staphylococcus	yes
2.0e+01	2.5e+00	5.4e-03	1.4e-02	ACC - Q5	30x	Otu0004	Staphylococcus	yes
2.0e+01	-2.7e+00	3.0e-03	1.5e-02	K - PL	30x	Otu0001	Salmonella	yes
2.0e+01	-2.5e+00	5.4e-03	1.8e-02	ACC - Q5	30x	Otu0001	Salmonella	yes
2.0e+01	2.2e+00	1.2e-02	3.1e-02	PL - Q5	30x	Otu0001	Salmonella	yes
2.0e+01	-2.1e+00	1.9e-02	3.2e-02	ACC - K	30x	Otu0001	Salmonella	yes
2.0e+01	2.1e+00	1.7e-02	3.4e-02	K - PHU	30x	Otu0004	Staphylococcus	yes
2.0e+01	-2.1e+00	1.7e-02	3.5e-02	PHU - PL	30x	Otu0001	Salmonella	yes
2.0e+01	2.0e+00	2.5e-02	4.2e-02	K - Q5	30x	Otu0004	Staphylococcus	yes

Table S8: Dunns post-hoc results of differences in model classification success using 16S rRNA gene sequencing data

Chi Sq	Z	P-value	BH	Sample Type 1	Model 1	Sample Type 2	Model 2
4.8e+02	4.9e+00	4.9e-07	5.5e-07	fecal	cycles	fecal	polymerase
4.8e+02	-1.5e+01	4.2e-49	2.1e-48	fecal	cycles	fecal	individual
4.8e+02	-2.0e+01	1.2e-85	1.2e-84	fecal	polymerase	fecal	individual
4.8e+02	-9.8e+00	6.5e-23	1.1e-22	fecal	cycles	mock	cycles
4.8e+02	-1.5e+01	4.3e-49	1.4e-48	fecal	polymerase	mock	cycles
4.8e+02	4.9e+00	4.9e-07	6.1e-07	fecal	individual	mock	cycles
4.8e+02	-4.9e+00	4.9e-07	7.0e-07	fecal	cycles	mock	polymerase
4.8e+02	-9.8e+00	6.2e-23	1.5e-22	fecal	polymerase	mock	polymerase
4.8e+02	9.8e+00	6.5e-23	1.3e-22	fecal	individual	mock	polymerase
4.8e+02	4.9e+00	5.1e-07	5.1e-07	mock	cycles	mock	polymerase