

# **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