

The Influence of Different High Fidelity DNA Polymerase on 16S rRNA Sequencing

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Supplemental

Table S1: ANOVA Results of Differences in Number of OTUs between HiFi DNA Polymerases in Fecal Samples

DF	Sum Squares	Mean Squares	F value	P-value	BH	Cycle	Sub-Sample Depth
4	3.66	0.91	1.25	3.5e-01	3.5e-01	20x	1000
4	6.90	1.73	2.84	6.1e-02	1.2e-01	25x	1000
3	4.67	1.56	2.55	1.0e-01	1.4e-01	30x	1000
4	13.01	3.25	16.35	2.4e-05	9.7e-05	35x	1000
4	5.15	1.29	4.57	1.2e-01	1.2e-01	20x	5000
4	8.11	2.03	4.98	1.3e-02	1.8e-02	25x	5000
3	10.36	3.45	25.24	1.8e-05	3.6e-05	30x	5000
4	14.38	3.59	81.00	1.6e-09	6.4e-09	35x	5000
2	2.85	1.43	3.73	1.5e-01	1.5e-01	20x	10000
4	10.39	2.60	11.92	3.8e-04	5.1e-04	25x	10000
3	11.22	3.74	57.90	2.1e-07	4.2e-07	30x	10000
4	13.25	3.31	57.20	4.0e-08	1.6e-07	35x	10000
2	0.27	0.13	0.10	9.1e-01	9.1e-01	20x	15000
4	8.69	2.17	7.23	4.1e-03	5.5e-03	25x	15000
3	10.40	3.47	25.95	1.6e-05	3.1e-05	30x	15000
4	12.24	3.06	48.03	2.7e-07	1.1e-06	35x	15000
1	1.00	1.00	NA	NA	NA	20x	20000
4	9.84	2.46	12.50	4.5e-04	4.5e-04	25x	20000
3	10.65	3.55	31.67	5.5e-06	8.3e-06	30x	20000
3	11.61	3.87	119.82	3.3e-09	9.9e-09	35x	20000

Table S2: Tukey Post-Hoc Results of Differences in Number of OTUs between HiFi DNA Polymerases in Fecal Samples

Difference	Lower	Upper	P Adjusted	Comparison	Cycle	Sub-Sample Depth
299.50	88.49	510.51	5.7e-03	PL-ACC	35x	20000
192.50	53.41	331.59	5.8e-03	PL-ACC	35x	10000
251.75	52.99	450.51	1.2e-02	PL-ACC	35x	15000
119.92	14.60	225.23	2.3e-02	PL-K	35x	5000
155.50	16.41	294.59	2.6e-02	PL-PHU	35x	10000
108.00	10.50	205.50	2.7e-02	PL-ACC	35x	5000
235.75	24.74	446.76	2.7e-02	PL-PHU	35x	20000
187.00	16.65	357.35	2.9e-02	PL-K	35x	10000

Table S3: ANOVA Results of Differences in Number of OTUs between HiFi DNA Polymerases in Mock Samples

DF	Sum Squares	Mean Squares	F value	P-value	BH	Cycle	Sub-Sample Depth
2	364.56	182.28	15.82	1.7e-03	2.2e-03	20x	1000
4	3478.53	869.63	75.15	2.7e-09	1.1e-08	25x	1000
4	2206.58	551.64	10.28	1.8e-05	3.6e-05	30x	1000
4	1971.92	492.98	2.31	1.0e-01	1.0e-01	35x	1000
3	267.19	89.06	9.44	1.8e-03	2.0e-03	25x	5000
3	7731.36	2577.12	80.88	1.1e-12	3.3e-12	30x	5000
3	13086.29	4362.10	8.67	2.0e-03	2.0e-03	35x	5000
3	400.50	133.50	7.14	5.2e-03	5.2e-03	25x	10000
3	18644.56	6214.85	115.99	5.0e-14	1.5e-13	30x	10000
3	50519.50	16839.83	435.98	1.6e-12	2.5e-12	35x	10000

Table S4: Tukey Post-Hoc Results of Differences in Number of OTUs between HiFi DNA Polymerases in Mock Samples

Difference	Lower	Upper	P Adjusted	Comparison	Cycle	Sub-Sample Depth
75.85	63.87	87.83	6.6e-14	PL-ACC	30x	10000
142.50	129.45	155.55	1.9e-12	PL-ACC	35x	10000
46.95	37.74	56.16	2.6e-12	PL-ACC	30x	5000
129.25	116.20	142.30	4.0e-12	PL-PHU	35x	10000
-108.25	-121.30	-95.20	4.6e-11	Q5-PL	35x	10000
-36.77	-46.07	-27.46	8.1e-10	Q5-PL	30x	10000
44.35	32.37	56.33	2.8e-09	PL-PHU	30x	10000
33.20	23.99	42.41	3.2e-09	PL-PHU	30x	5000
-38.50	-46.60	-30.40	5.1e-09	Q5-K	25x	1000
-37.50	-45.60	-29.40	7.3e-09	PHU-K	25x	1000
-23.60	-30.56	-16.64	1.0e-08	Q5-PL	30x	5000
36.50	28.40	44.60	1.1e-08	K-ACC	25x	1000
-35.50	-43.60	-27.40	1.5e-08	PL-K	25x	1000
39.08	26.91	51.26	3.9e-08	Q5-ACC	30x	10000
23.35	14.14	32.56	1.8e-06	Q5-ACC	30x	5000
31.50	17.18	45.82	1.9e-05	PHU-ACC	30x	10000
34.25	21.20	47.30	2.5e-05	Q5-ACC	35x	10000
22.03	9.31	34.74	1.8e-04	K-ACC	30x	1000
-15.48	-25.20	-5.75	5.7e-04	Q5-K	30x	1000
-13.50	-20.36	-6.64	1.3e-03	PL-PHU	20x	1000
-18.78	-31.49	-6.06	1.5e-03	PHU-K	30x	1000
21.00	7.95	34.05	2.2e-03	Q5-PHU	35x	10000
67.90	23.72	112.08	2.9e-03	PL-ACC	35x	5000

Difference	Lower	Upper	P Adjusted	Comparison	Cycle	Sub-Sample Depth
9.75	3.30	16.20	3.6e-03	PL-PHU	25x	5000
9.50	3.05	15.95	4.3e-03	PL-ACC	25x	5000
61.90	17.72	106.08	5.9e-03	PL-PHU	35x	5000
-9.00	-15.45	-2.55	6.4e-03	Q5-PL	25x	5000
-12.25	-21.33	-3.17	8.2e-03	Q5-PL	25x	10000
15.55	3.03	28.07	9.0e-03	PL-ACC	30x	1000
12.00	2.92	21.08	9.4e-03	PL-PHU	25x	10000
13.75	2.74	24.76	1.1e-02	PHU-ACC	30x	5000
9.75	0.67	18.83	3.4e-02	PL-ACC	25x	10000
9.60	0.39	18.81	3.9e-02	Q5-PHU	30x	5000
13.25	0.20	26.30	4.6e-02	PHU-ACC	35x	10000

Table S5: Kruskal-Wallis Results of Differences in Per Base Error Rate between HiFi DNA Polymerases in Mock Samples

Rank Sum Statistic	DF	P-value	BH	Cycle	Sub-Sample Depth
7.85	2	2.0e-02	2.0e-02	20x	full_data
12.95	4	1.2e-02	1.5e-02	25x	full_data
28.31	4	1.1e-05	4.3e-05	30x	full_data
15.00	4	4.7e-03	9.4e-03	35x	full_data

Table S6: Dunn's Post-Hoc Results of Differences in Per Base Error Rate between HiFi DNA Polymerases in Mock Samples

Chi Squared	Z Statistic	P Adjusted	Comparison	Cycle	Sub-Sample Depth
28.31	-4.46	4.1e-06	ACC - K	30x	full_data
28.31	4.32	7.7e-06	K - PL	30x	full_data
12.95	3.26	5.6e-04	K - Q5	25x	full_data
15.00	3.14	8.6e-04	K - PHU	35x	full_data
15.00	-3.05	1.2e-03	PHU - PL	35x	full_data
7.85	2.77	2.8e-03	PHU - PL	20x	full_data
28.31	-2.69	3.6e-03	ACC - Q5	30x	full_data
12.95	2.68	3.7e-03	K - PHU	25x	full_data
28.31	2.65	4.0e-03	K - PHU	30x	full_data
28.31	2.37	8.8e-03	K - Q5	30x	full_data
15.00	-2.33	1.0e-02	ACC - K	35x	full_data
15.00	-2.05	2.0e-02	ACC - PL	35x	full_data
12.95	2.01	2.2e-02	ACC - Q5	25x	full_data
28.31	-2.00	2.3e-02	PL - Q5	30x	full_data
12.95	1.88	3.0e-02	PL - Q5	25x	full_data
15.00	-1.79	3.7e-02	PHU - Q5	35x	full_data
15.00	1.68	4.7e-02	K - Q5	35x	full_data
7.85	-1.64	5.0e-02	PL - Q5	20x	full_data

Table S7: Kruskal-Wallis Results of Differences in Sequences with Error between HiFi DNA Polymerases in Mock Samples

Rank Sum Statistic	DF	P-value	BH	Cycle	Sub-Sample Depth
7.85	2	2.0e-02	2.0e-02	20x	full_data
15.98	4	3.0e-03	5.2e-03	25x	full_data
29.22	4	7.0e-06	2.8e-05	30x	full_data
15.44	4	3.9e-03	5.2e-03	35x	full_data

Table S8: Dunn's Post-Hoc Results of Differences in Sequences with Error between HiFi DNA Polymerases in Mock Samples

Chi Squared	Z Statistic	P Adjusted	Comparison	Cycle	Sub-Sample Depth
29.22	4.17	1.5e-05	K - Q5	30x	full_data
29.22	3.89	5.0e-05	K - PHU	30x	full_data
15.44	-3.71	1.0e-04	PHU - PL	35x	full_data
29.22	3.57	1.8e-04	PL - Q5	30x	full_data
29.22	-3.41	3.2e-04	PHU - PL	30x	full_data
15.98	3.37	3.7e-04	K - Q5	25x	full_data
15.98	2.79	2.6e-03	K - PHU	25x	full_data
7.85	2.77	2.8e-03	PHU - PL	20x	full_data
15.98	2.76	2.9e-03	ACC - Q5	25x	full_data
15.44	2.38	8.6e-03	PL - Q5	35x	full_data
15.44	2.38	8.7e-03	K - PHU	35x	full_data
15.98	2.14	1.6e-02	ACC - PHU	25x	full_data
15.98	1.86	3.1e-02	K - PL	25x	full_data
15.44	1.85	3.2e-02	ACC - PHU	35x	full_data
15.44	-1.85	3.2e-02	ACC - PL	35x	full_data
29.22	-1.81	3.5e-02	ACC - K	30x	full_data
29.22	1.76	3.9e-02	ACC - PHU	30x	full_data
7.85	-1.64	5.0e-02	PL - Q5	20x	full_data

Table S9: Kruskal-Wallis Results of Differences in Chimera Prevalence between HiFi DNA Polymerases in Mock Samples

Rank Sum Statistic	DF	P-value	BH	Cycle	Sub-Sample Depth
8.91	2	1.2e-02	1.2e-02	20x	full_data
15.54	4	3.7e-03	4.9e-03	25x	full_data
21.41	4	2.6e-04	1.1e-03	30x	full_data
16.28	4	2.7e-03	4.9e-03	35x	full_data

Table S10: Dunn's Post-Hoc Results of Differences in Chimera Prevalence between HiFi DNA Polymerase in Mock Samples

Chi Squared	Z Statistic	P Adjusted	Comparison	Cycle	Sub-Sample Depth
21.41	-4.23	1.2e-05	ACC - PL	30x	full_data
16.28	-3.71	1.0e-04	ACC - PL	35x	full_data
15.54	3.37	3.7e-04	K - PHU	25x	full_data
8.91	2.98	1.4e-03	PHU - PL	20x	full_data
21.41	2.89	1.9e-03	PL - Q5	30x	full_data
21.41	-2.84	2.2e-03	ACC - K	30x	full_data
15.54	-2.76	2.9e-03	PHU - PL	25x	full_data
15.54	-2.68	3.7e-03	ACC - K	25x	full_data
16.28	-2.65	4.0e-03	PHU - PL	35x	full_data
21.41	-2.59	4.8e-03	PHU - PL	30x	full_data
16.28	-2.38	8.7e-03	ACC - K	35x	full_data
16.28	-2.12	1.7e-02	ACC - Q5	35x	full_data
21.41	-2.05	2.0e-02	ACC - Q5	30x	full_data
15.54	-2.01	2.2e-02	ACC - PL	25x	full_data
15.54	1.98	2.4e-02	K - Q5	25x	full_data
21.41	-1.73	4.2e-02	K - PL	30x	full_data