Electronic Supplementary Material (ESI) for Food & Function.

DNA-sequencing report

(This document is subject to change as required)

NCGM_project no (Suflam ID):	**NCGM-201**
Customer Details	
Project Head Name:	Dr. Vikram Patial
Contact person Email:	vikrampatial@ihbt.res.in
Institute:	CSIR-IHBT
Sample Received date:	26/02/2021
Final Report date:	13-04-2021(Partial Report) 07-6-2021 (final report)
Delivery date:	13-04-2021(Partial Report)
Project Details	
NGS service:	G.16s
Species:	Rat Fecal
Number of samples:	18
Sequencing parameters:	Ion 540
Platform used:	Ion Torrent
Sequencing Depth asked:	0.15GB/Sample
Sequencing depth delivered:	> 0.15 GB (17 samples), 1 sample resequencing

Objective of this Report:

This report summarizes the DNA-seq services performed at NCGM. In-depth protocol will be available upon request. The report contains the information on Sample QC (Section-A), Library QC (Section-B) and standard Bioinformatics analysis (Section-C).

Contact person for the report:

Name: Khushbu Patel
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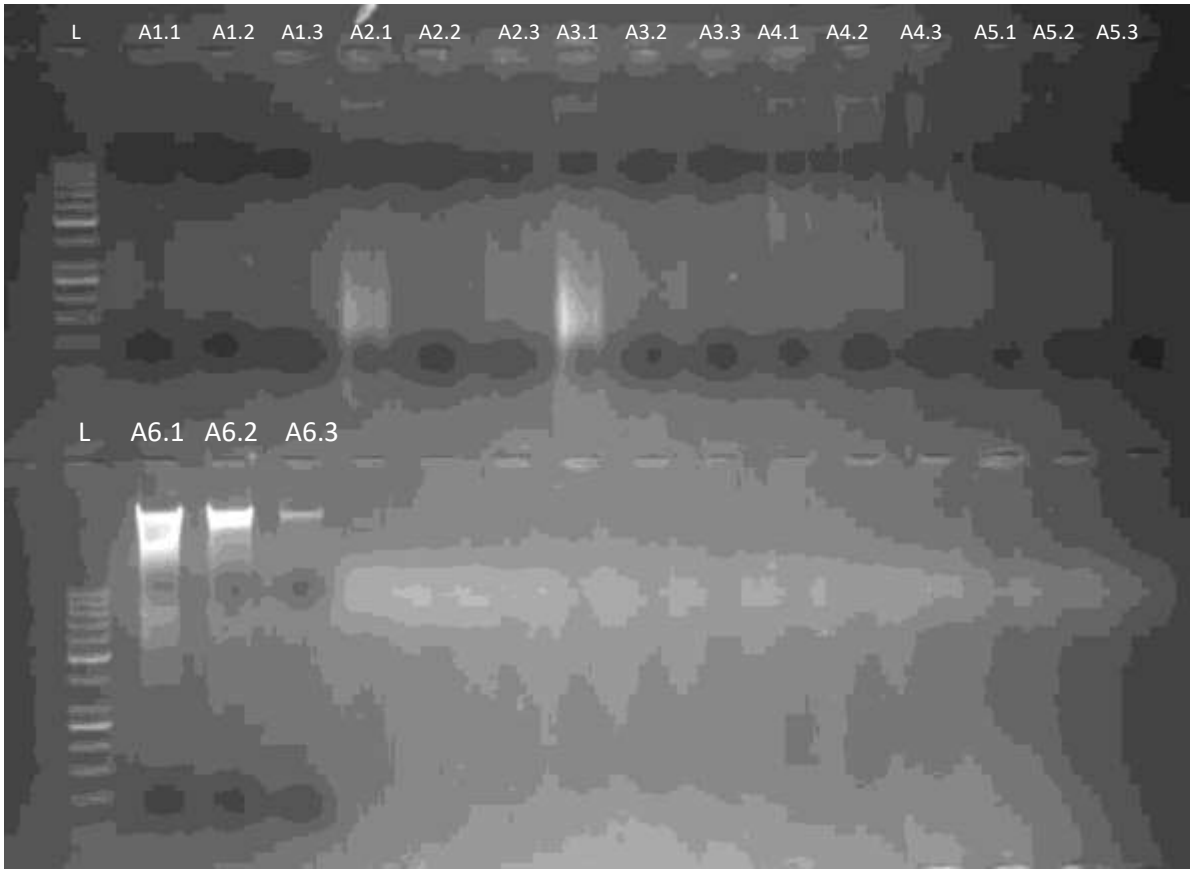
Section-A

Sample QC report

DNA quantity check: Extracted DNA quantity ischeck with the help of nanodrop 1000, average of two individual data points is provided below in the table.

SR NO	SAMPLE ID	SAMPLE NAME	ng/ul	A260/280	A260/230	QC remarks
1	10200150855	A1.1	32.1	1.71	1.58	Pass
2	10200150858	A1.2	96.4	1.93	1.72	Pass
3	10200150863	A1.3	17.7	2.02	2.11	Pass
4	10200150889	A2.1	597.8	2.23	2.4	Pass
5	10200150892	A2.2	27.5	2.08	1.57	Pass
6	10200150894	A2.3	84.3	2.1	1.72	Pass
7	10200150895	A3.1	498.4	2.16	1.98	Pass
8	10200150899	A3.2	25.9	1.62	1.35	Pass
9	10200150901	A3.3	12.4	1.58	1.32	Pass
10	10200150906	A4.1	150.4	2.09	1.9	Pass
11	10200150909	A4.2	57.8	2.12	1.37	Pass
12	10200150910	A4.3	136.4	2.12	1.71	Pass
13	10200150913	A5.1	29.1	2.13	1.31	Pass
14	10200150916	A5.2	91.8	2.15	1.53	Pass
15	10200150917	A5.3	99.1	2.2	1.59	Pass
16	10200150920	A6.1	1028	1.9	2.1	Pass
17	10200150921	A6.2	174	1.89	1.7	Pass
18	10200150923	A6.3	74.4	2.15	1.47	Pass

DNA Quality check: The quality of the quantified DNA was confirmed on the 1% agarose gel. In brief, 2 uls of the DNA is mixed with 2 uls of 6x Loading dye (Invitrogen) and subjected to electrophoresis at 120 volts for 30 mins. Scanned gel image is embedded below.



Conclusion: All Samples are considered pass.

Section-B

Library QC report

B.1 Amplification of 16S hypervariable region

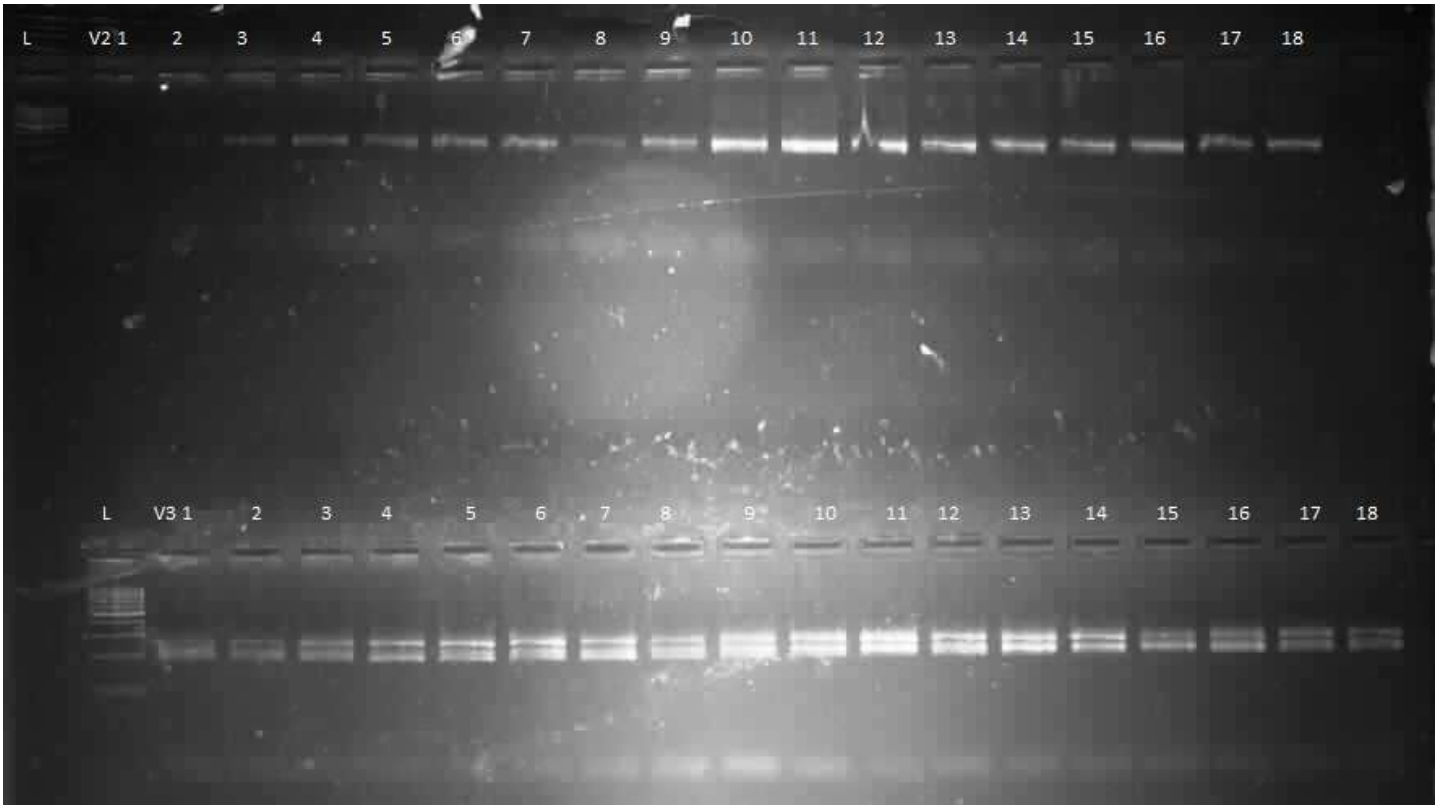
PCR template setup reaction (Ion 16S Metagenomics Kit)

Component	V2-4	V3-6, 7-9
2X environmental Mastermix	15 ul	15 ul
16S primer set	3 uls	3 uls
Template DNA	5 uls (100 ng)	5 uls (100 ng)
Nuclease-free Water	7 uls	7 uls
Total	30 uls	30 uls

PCR Amplification conditions:

Stage	Temperature	Time
Hold	95 C	10 min
Cycles 25	95 C	30 sec
	58 C	30 sec
	72 C	20 sec
Hold	72 C	7 min
Hold	4 C	Infinite

Observation: Amplicons quality post PCR cleanup. We used 2 uls of PCR product on 1% agarose gel and gel images are embedded for the reference. Control DNA (E.coli gDNA) provided in the kit used for the reference of the amplicons. e have used highQu Next-gen 100 bp DNA ladder on the gel as a marker.



Top Layer: Samples 1-18 amplicons obtained from V2-4 primer set.

Bottom Layer: Samples 1-18 amplicons obtained from V3-6/7-9 primers set.

Conclusion: Amplified PCR products will be utilized further for the library preparation.

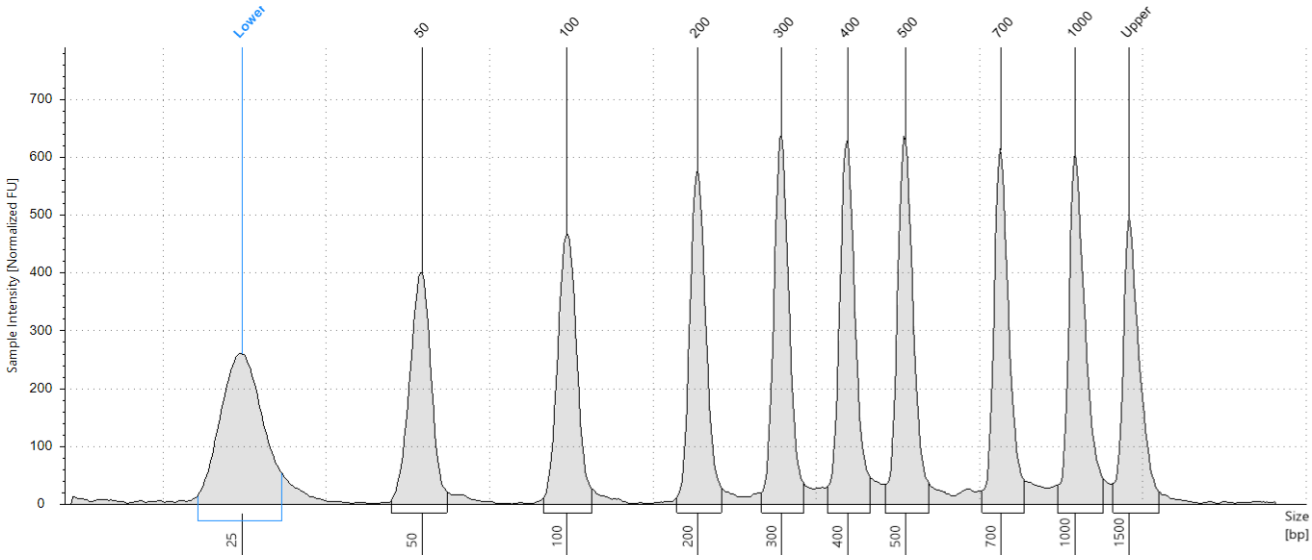
Library preparation and QC: Final libraries were quantified using Qubit 4.0 fluorometer (Thermofisher #Q33238) using DNA HS assay kit (Thermofisher #Q32851) following manufacturer's protocol. Obtained results are presented below in the table. To identify the insert size of the library, we queried it on Tapestation 4150 (Agilent) utilizing high sensitive D1000 screentapes (Agilent # 5067-5582) following manufacturers' protocol. Acquired sizes of all libraries are reported in the following table.

SR. NO	Lab Id	Sample name	Quant (ng/ul)	Index	QC remarks
1	10200150855	A1.1	5.24	1	Pass
2	10200150858	A1.2	5.08	2	Pass
3	10200150863	A1.3	7.26	3	Pass
4	10200150889	A2.1	9.1	4	Pass
5	10200150892	A2.2	8.7	5	Pass
6	10200150894	A2.3	12.9	6	Pass
7	10200150895	A3.1	9.42	7	Pass
8	10200150899	A3.2	3.04	8	Pass
9	10200150901	A3.3	5.86	9	Pass
10	10200150906	A4.1	15.5	10	Pass
11	10200150909	A4.2	15.6	11	Pass
12	10200150910	A4.3	8	12	Pass
13	10200150913	A5.1	10	13	Pass
14	10200150916	A5.2	10.1	14	Pass
15	10200150917	A5.3	8.98	15	Pass
16	10200150920	A6.1	5.46	16	Pass
17	10200150921	A6.2	5.8	17	Pass
18	10200150923	A6.3	4.66	18	Pass

Conclusion: All libraries are considered pass and will be removed from adapter contamination before sequencing.

Filename: 2021-03-24 - 17.42.36.HSD1000

EL1: Electronic Ladder



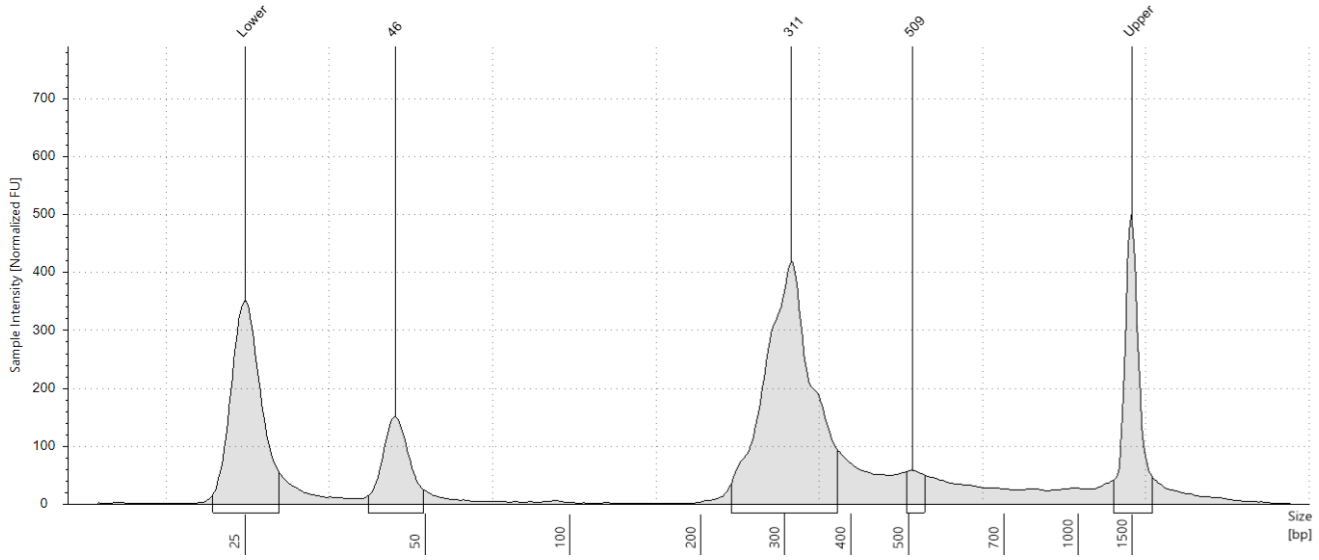
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
EL1	2350	Electronic Ladder		Ladder

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	340	-	20900	-	
50	265	-	8160	11.28	
100	278	-	4270	11.82	
200	290	-	2230	12.32	
300	304	-	1560	12.95	
400	306	-	1180	13.00	
500	312	-	961	13.29	
700	286	-	629	12.19	
1000	309	-	476	13.15	
1500	250	250	256	-	

A1: A1.1



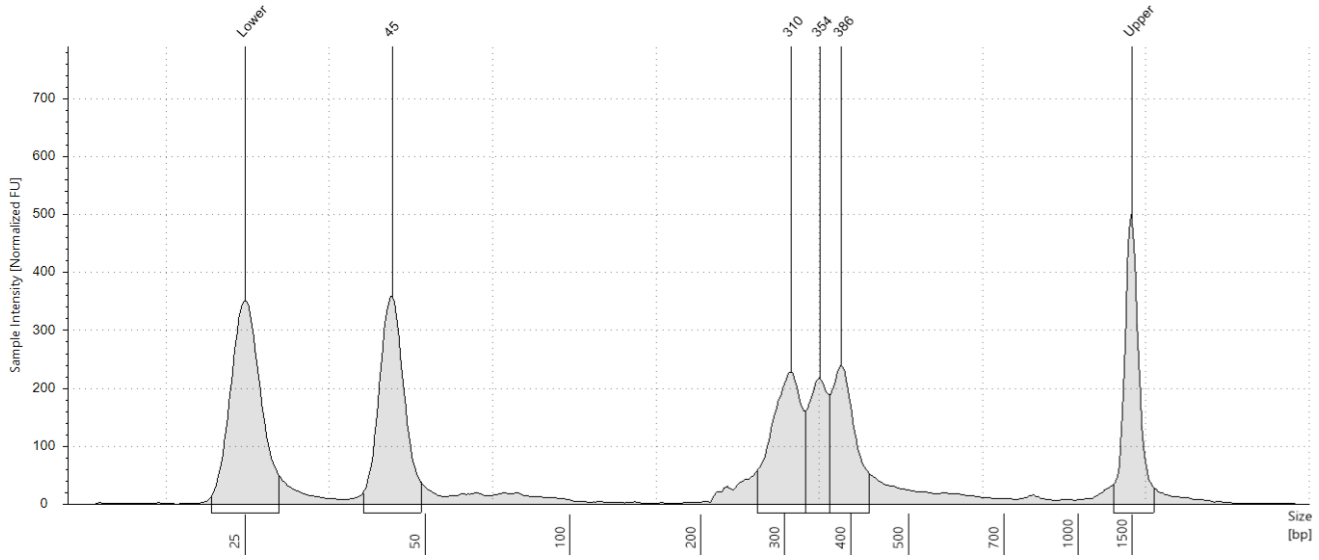
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A1	857	A1.1		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	368	-	22700	-	
46	138	-	4620	16.06	
311	687	-	3400	80.18	
509	32.2	-	97.2	3.75	
1500	250	250	256	-	

B1: A1.2



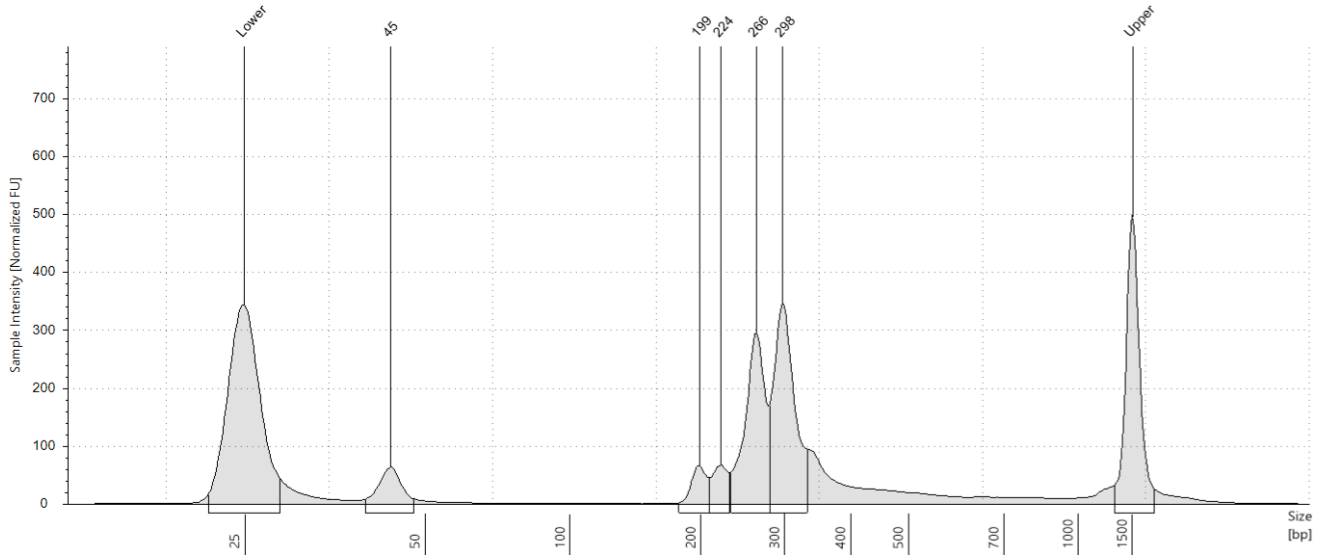
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
B1	904	A1.2		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	375	-	23100	-	
45	317	-	10800	35.08	
310	238	-	1180	26.34	
354	151	-	656	16.68	
386	198	-	788	21.90	
1500	250	250	256	-	

C1: A1.3



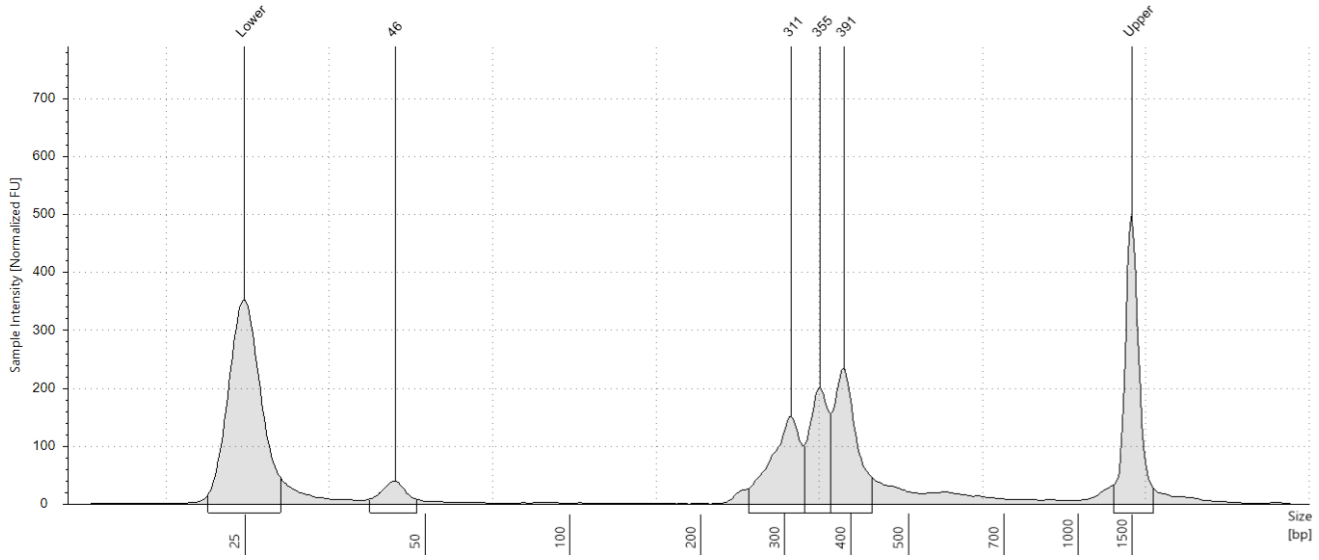
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
C1	608	A1.3		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	406	-	25000	-	
45	51.2	-	1740	8.42	
199	36.0	-	279	5.92	
224	39.3	-	270	6.47	
266	217	-	1250	35.62	
298	265	-	1370	43.57	
1500	250	250	256	-	

D1: A2.1



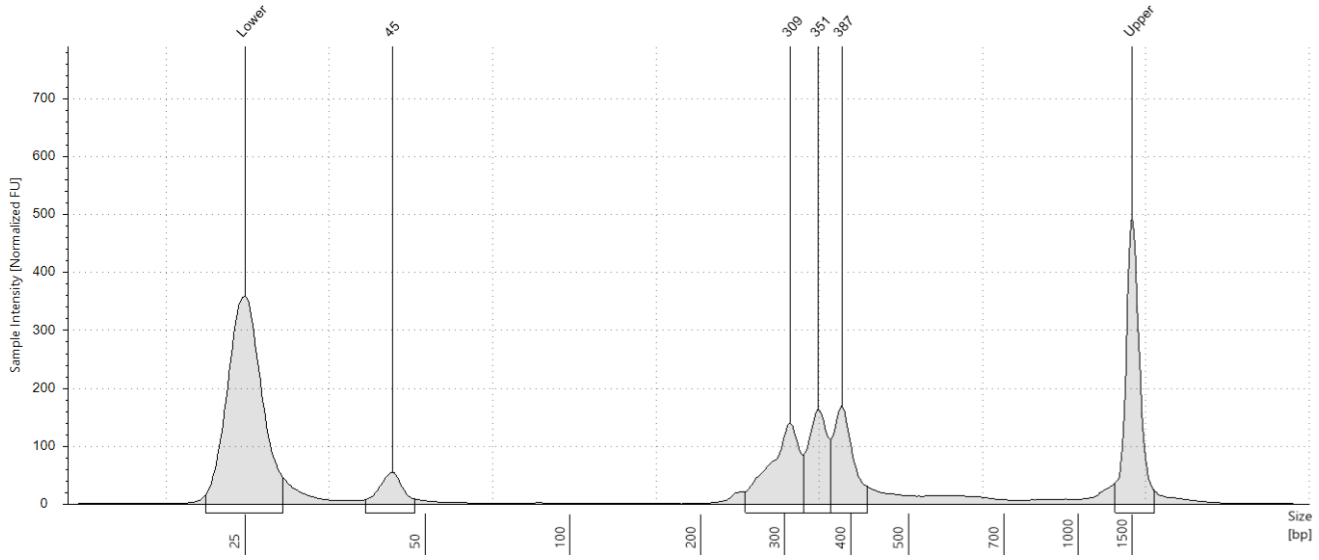
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
D1	518	A2.1		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	412	-	25400	-	
46	32.1	-	1070	6.19	
311	157	-	776	30.26	
355	140	-	607	27.04	
391	189	-	744	36.51	
1500	250	250	256	-	

E1: A2.2



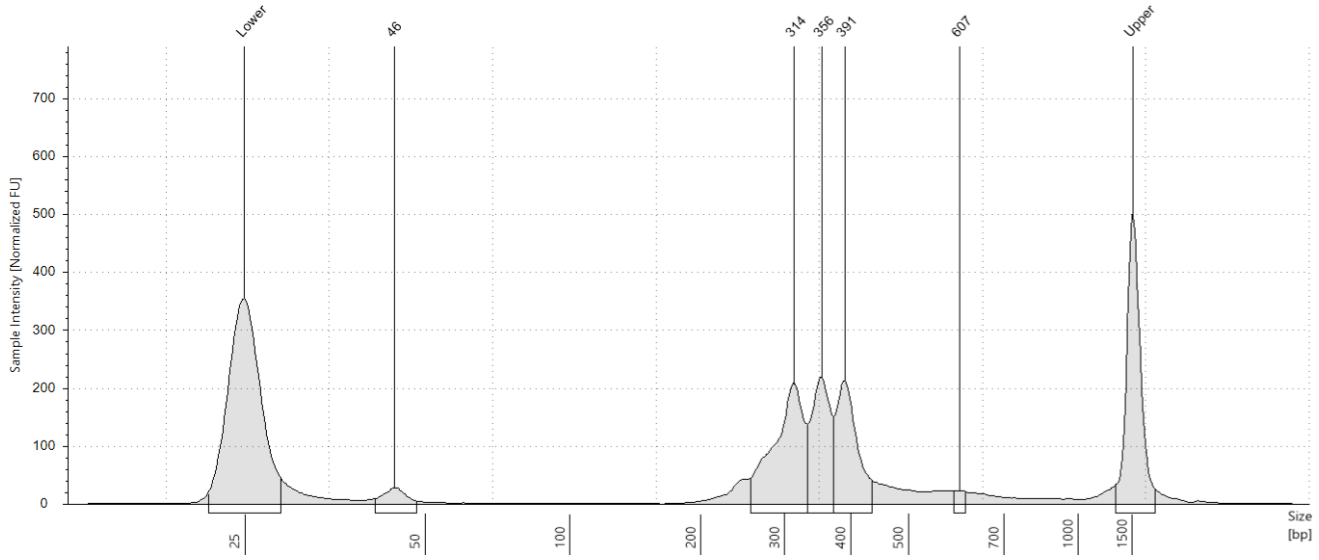
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
E1	434	A2.2		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	459	-	28200	-	
45	45.8	-	1550	10.55	
309	149	-	741	34.26	
351	116	-	508	26.73	
387	124	-	492	28.46	
1500	250	250	256	-	

F1: A2.3



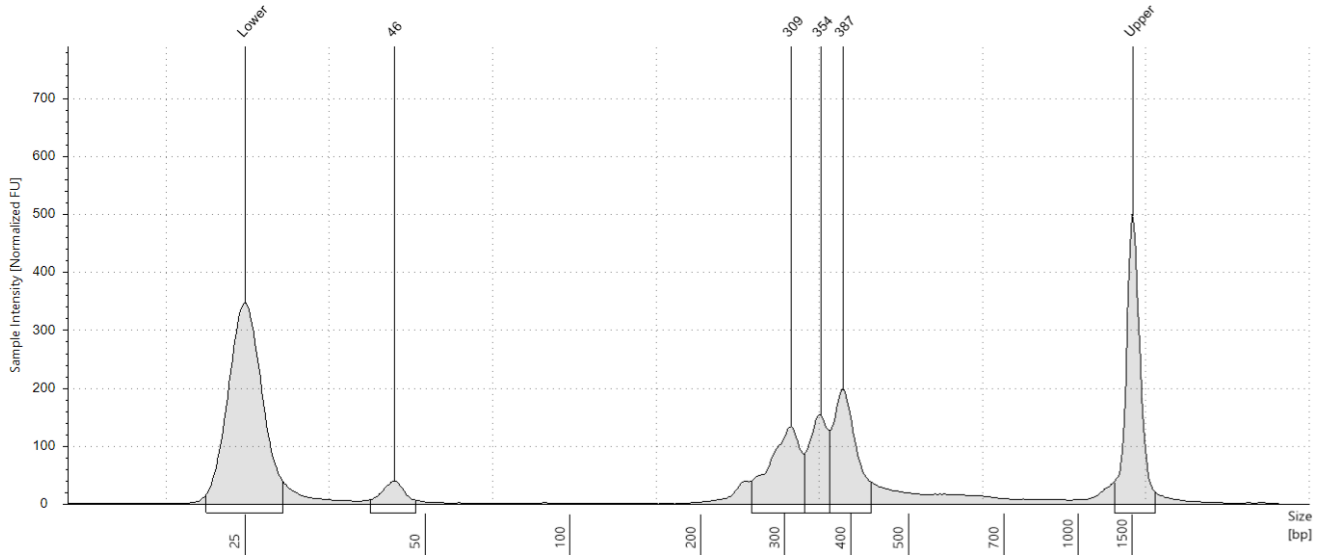
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
F1	571	A2.3		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	420	-	25900	-	
46	22.2	-	746	3.89	
314	220	-	1080	38.58	
356	156	-	673	27.30	
391	163	-	643	28.62	
607	9.21	-	23.3	1.61	
1500	250	250	256	-	

G1: A3.1



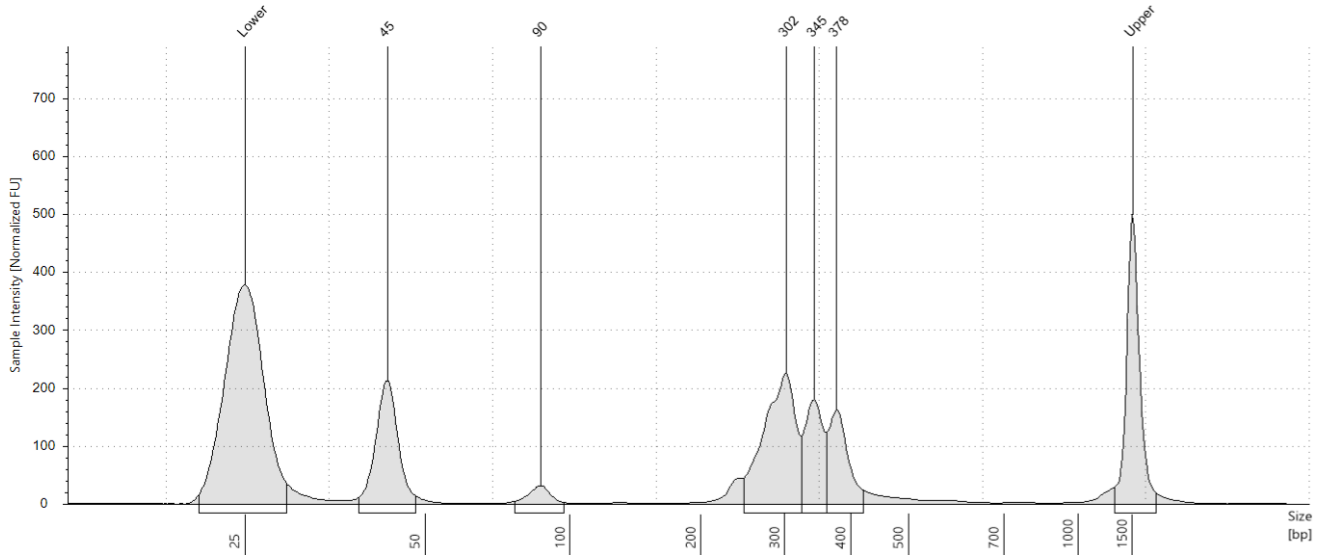
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
G1	447	A3.1		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	428	-	26300	-	
46	31.5	-	1060	7.05	
309	146	-	729	32.71	
354	103	-	449	23.05	
387	166	-	661	37.20	
1500	250	250	256	-	

H1: A3.2



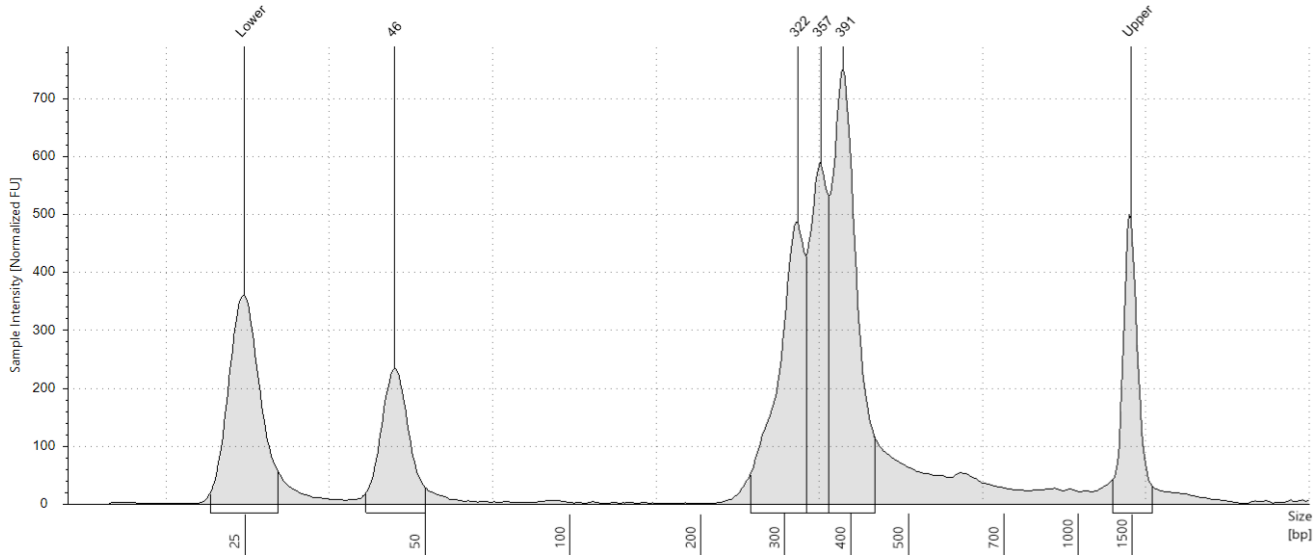
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
H1	736	A3.2		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	572	-	35200	-	
45	181	-	6240	24.59	
90	26.0	-	447	3.54	
302	276	-	1410	37.44	
345	128	-	571	17.39	
378	125	-	511	17.04	
1500	250	250	256	-	

A2: A3.3



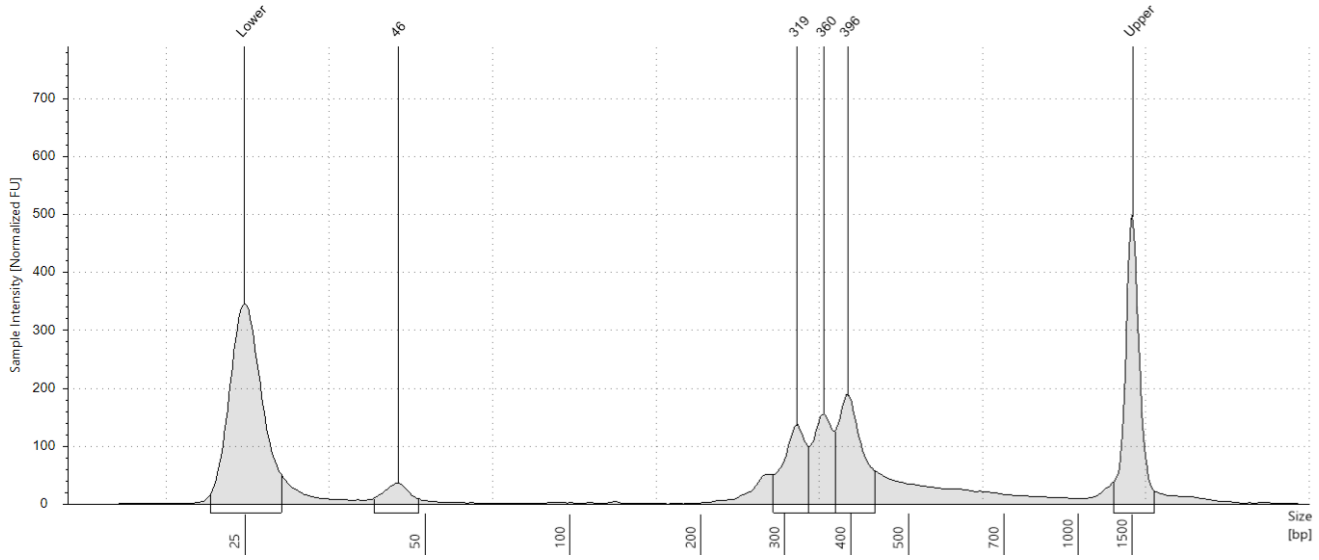
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A2	1650	A3.3		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	383	-	23600	-	
46	213	-	7140	12.93	
322	431	-	2060	26.16	
357	373	-	1610	22.62	
391	632	-	2480	38.29	
1500	250	250	256	-	

B2: A4.1



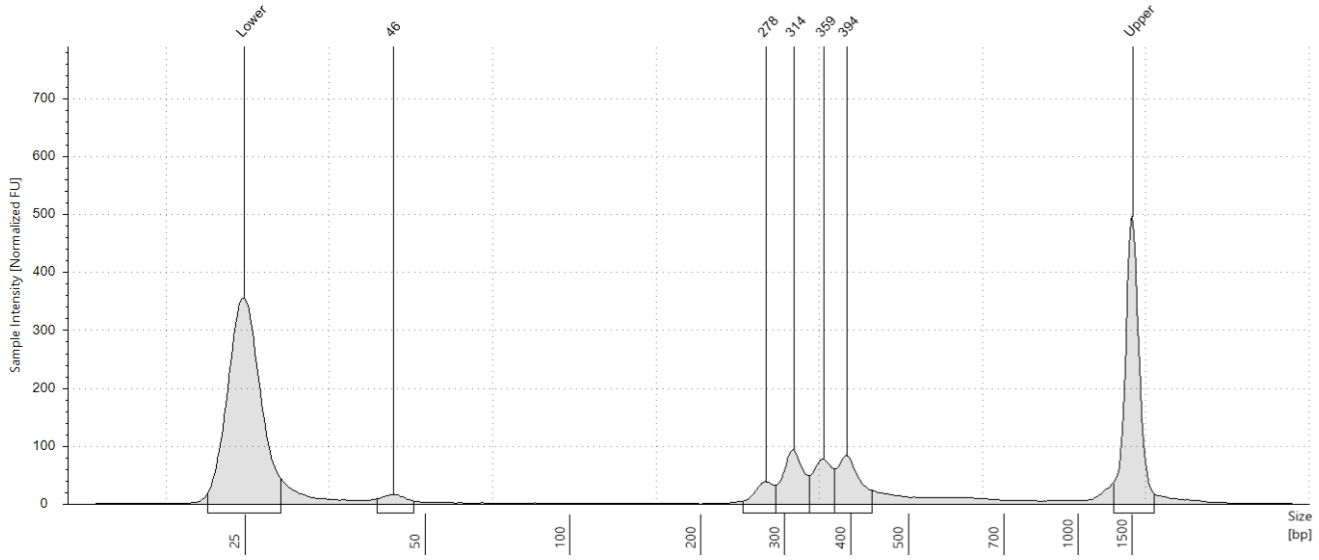
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
B2	414	A4.1		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	399	-	24600	-	
46	31.8	-	1050	7.67	
319	109	-	528	26.40	
360	111	-	475	26.81	
396	162	-	630	39.12	
1500	250	250	256	-	

C2: A4.2



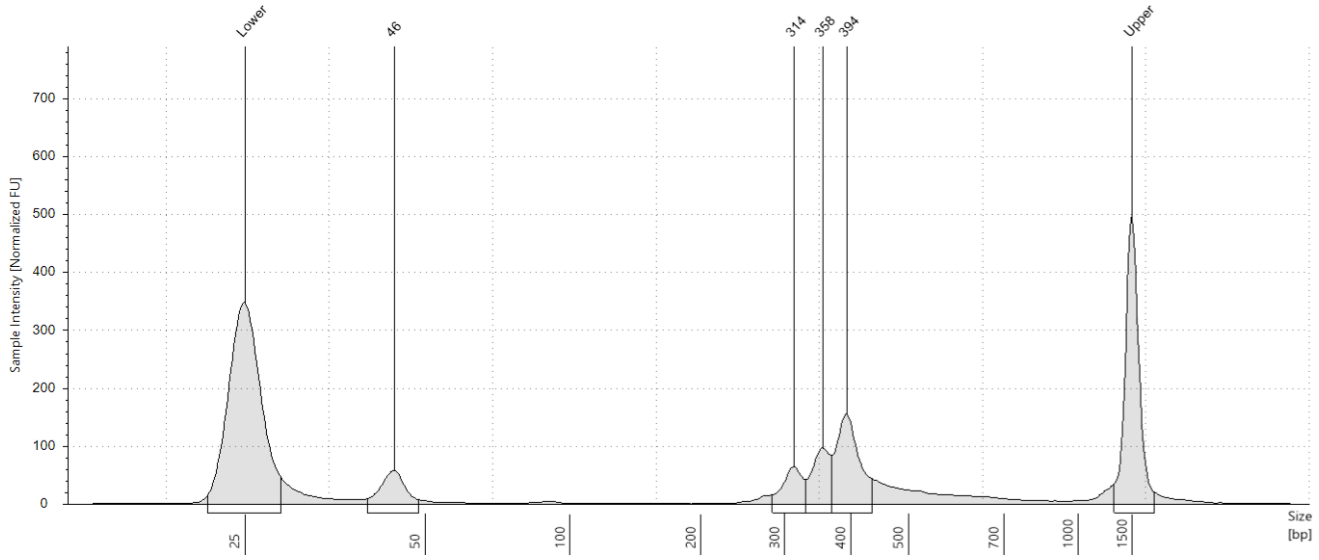
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
C2	228	A4.2		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	420	-	25900	-	
46	13.7	-	462	6.00	
278	25.6	-	142	11.23	
314	68.5	-	336	30.00	
359	53.9	-	231	23.59	
394	66.6	-	260	29.18	
1500	250	250	256	-	

D2: A4.3



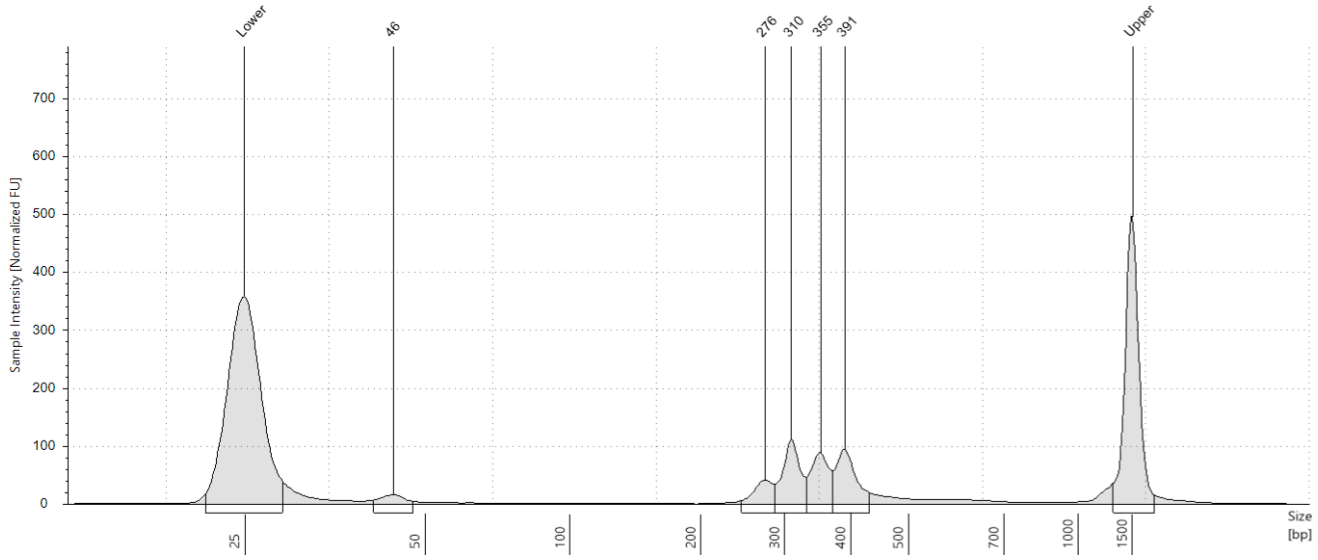
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
D2	291	A4.3		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	418	-	25700	-	
46	49.7	-	1670	17.07	
314	46.1	-	226	15.82	
358	63.5	-	273	21.79	
394	132	-	515	45.33	
1500	250	250	256	-	

E2: A5.1



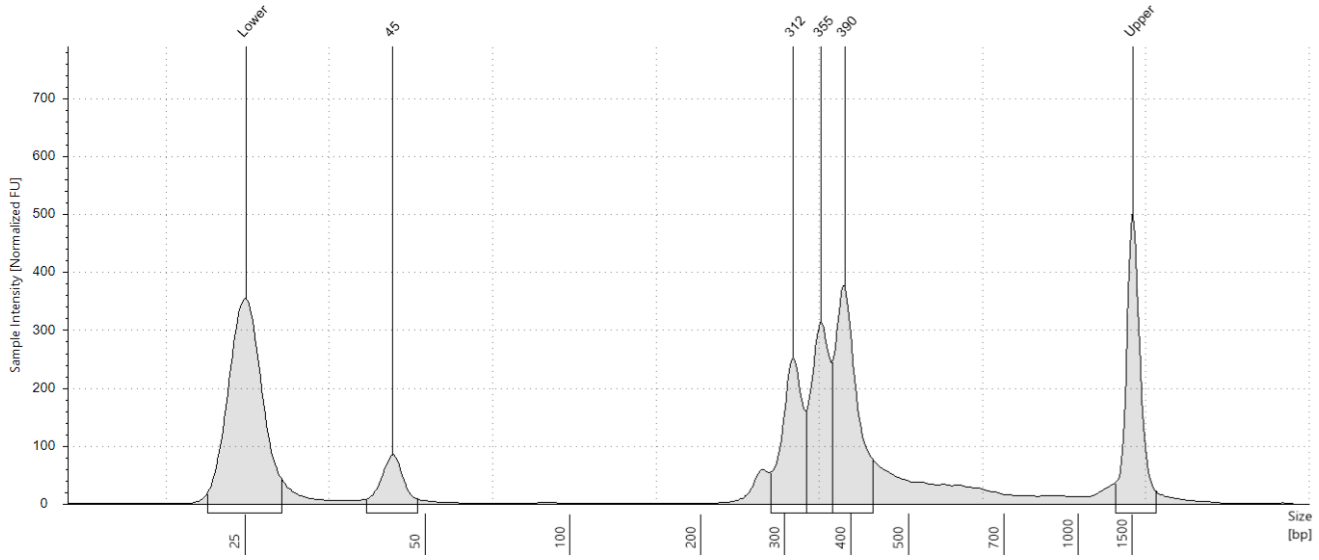
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
E2	239	A5.1		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	439	-	27000	-	
46	13.4	-	452	5.60	
276	27.9	-	155	11.66	
310	69.2	-	343	28.94	
355	60.3	-	262	25.22	
391	68.3	-	268	28.58	
1500	250	250	256	-	

F2: A5.2



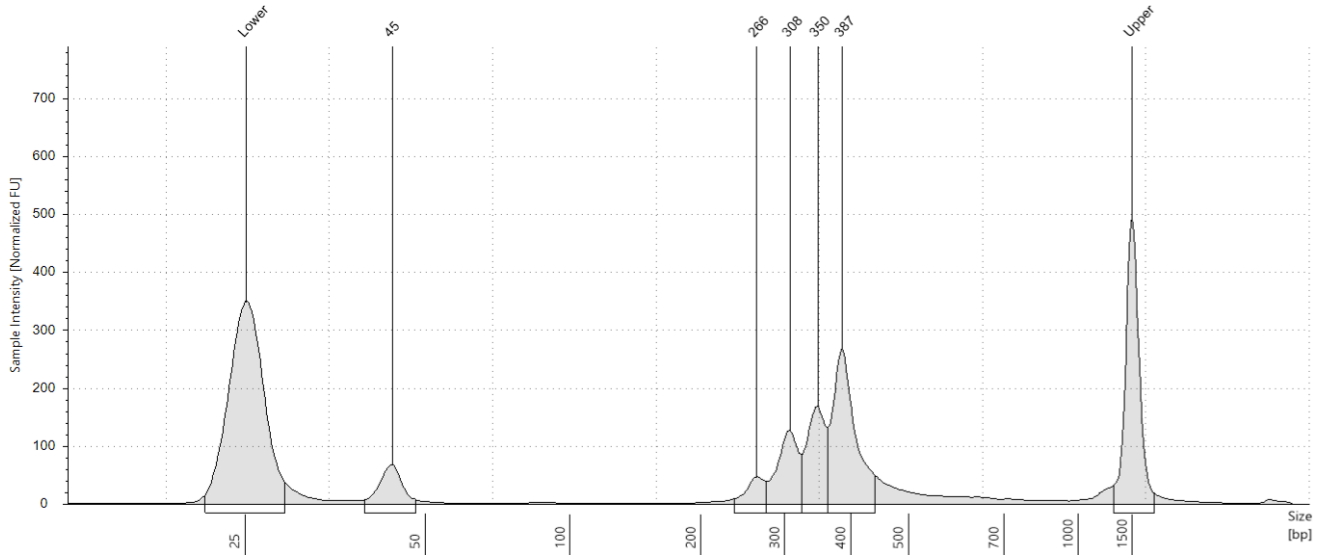
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
F2	765	A5.2		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	434	-	26700	-	
45	66.2	-	2240	8.66	
312	184	-	909	24.10	
355	216	-	935	28.21	
390	299	-	1180	39.04	
1500	250	250	256	-	

G2: A5.3



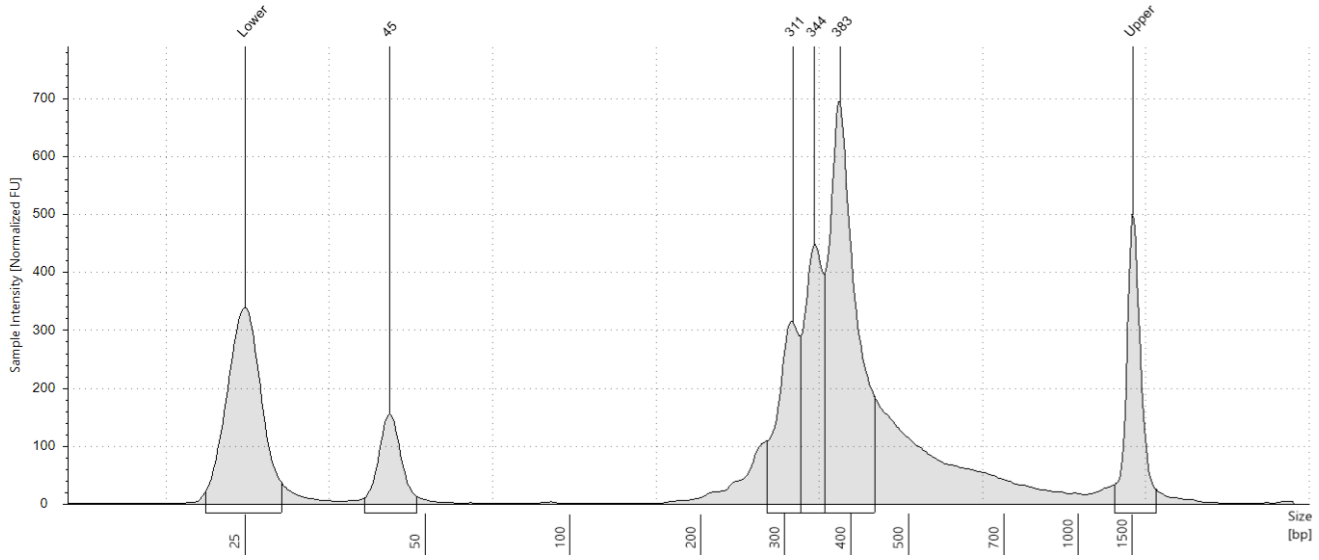
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
G2	531	A5.3		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	460	-	28300	-	
45	55.4	-	1880	10.42	
266	31.8	-	184	5.98	
308	98.2	-	491	18.48	
350	118	-	520	22.25	
387	228	-	905	42.86	
1500	250	250	256	-	

H2: A6.1



Sample Table

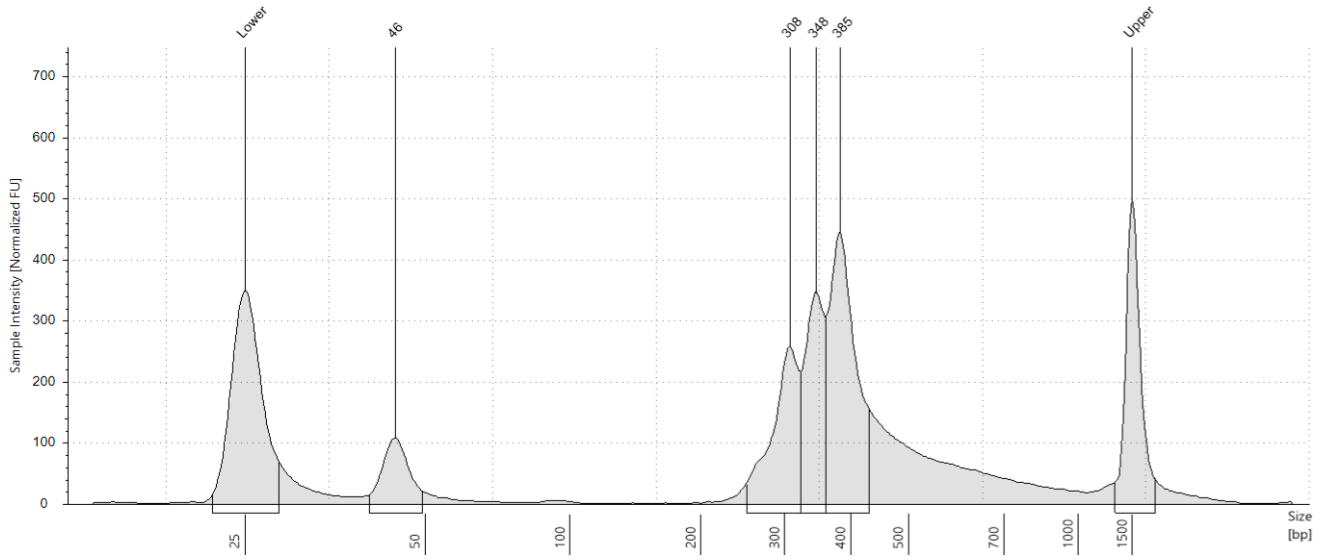
Well	Conc. [pg/µl]	Sample Description	Alert	Observations
H2	1350	A6.1		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	420	-	25800	-	
45	123	-	4200	9.12	
311	240	-	1190	17.83	
344	309	-	1380	22.91	
383	676	-	2710	50.14	
1500	250	250	256	-	

Filename: 2021-03-24 - 17.25.03.HSD1000

A1: A6.2



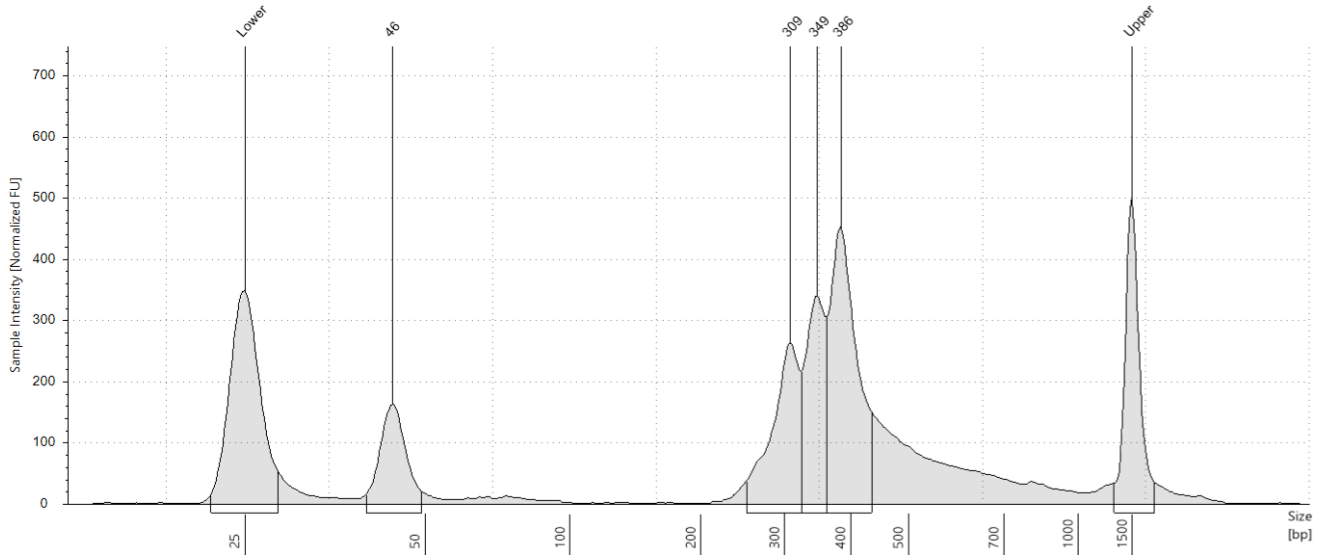
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A1	963	A6.2		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	366	-	22500	-	
46	95.7	-	3210	9.94	
308	229	-	1140	23.77	
348	233	-	1030	24.22	
385	405	-	1620	42.07	
1500	250	250	256	-	

B1: A6.3



Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
B1	1060	A6.3		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment
25	374	-	23000	-	
46	146	-	4930	13.71	
309	246	-	1230	23.16	
349	239	-	1050	22.48	
386	432	-	1720	40.65	
1500	250	250	256	-	

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Section – C
Sequencing Statistics

Sr. No	NCGM-No.	Lab ID	Sample Name	Index	Bases	Reads	Data in GB	Mean Read Length
1	NCGM-201	10200150855	A1.1	1	9,77,43,013	6,40,440	0.097743013	152
2	NCGM-201	10200150858	A1.2	2	36,25,69,949	19,51,394	0.362569949	185
3	NCGM-201	10200150863	A1.3	3	16,08,34,237	10,26,527	0.160834237	156
4	NCGM-201	10200150889	A2.1	4	45,28,38,544	24,93,151	0.452838544	181
5	NCGM-201	10200150892	A2.2	5	87,33,25,559	50,85,334	0.873325559	171
6	NCGM-201	10200150894	A2.3	6	83,80,17,459	48,05,883	0.838017459	174
7	NCGM-201	10200150895	A3.1	7	85,47,51,132	48,58,241	0.854751132	175
8	NCGM-201	10200150899	A3.2	8	85,98,03,193	46,61,168	0.859803193	184
9	NCGM-201	10200150901	A3.3	9	66,96,91,057	35,40,010	0.669691057	189
10	NCGM-201	10200150906	A4.1	10	52,78,13,793	31,54,097	0.527813793	167
11	NCGM-201	10200150909	A4.2	11	66,03,11,516	39,00,231	0.660311516	169
12	NCGM-201	10200150910	A4.3	12	73,05,64,790	40,67,912	0.73056479	179
13	NCGM-201	10200150913	A5.1	13	64,68,40,642	36,01,524	0.646840642	179
14	NCGM-201	10200150916	A5.2	14	61,15,23,979	33,80,123	0.611523979	180
15	NCGM-201	10200150917	A5.3	15	49,40,08,795	27,67,729	0.494008795	178
16	NCGM-201	10200150920	A6.1	16	86,62,20,893	52,48,066	0.866220893	165
17	NCGM-201	10200150921	A6.2	17	42,09,22,262	24,45,870	0.420922262	172
18	NCGM-201	10200150923	A6.3	18	45,13,28,149	25,83,593	0.451328149	174

Conclusion: Sample highlighted in yellow are considered for resequencing. Rest of the samples are finished in sequencing. Further individual analysis will be provided in a week time. For samples in resequencing and analysis will be completed in two weeks' time. If you need any specific type of analysis/grouping please provide information immediately.

1. SAMPLE PREPARATION:

Sequencing Platform: Ion Torrent.
Target Specification: 16S Ribosomal DNA gene sequence amplification

2. BIOINFORMATICS ANALYSIS

Details of the bioinformatics analysis are explained below:

2.1. Data statistics:

The data was generated on Ion Torrent platform with single end base pair chemistry. Statistics of the raw data is given in the table below:

Table 1: Statistics of raw data.

Sample Name	# of Reads	# of Bases	Max read length	Total data (MB)
A1.fastq	5,674,087	971,089,674	354	971
A2.fastq	**12,384,368**	**2,164,181,562**	**369**	**2,164**
A3.fastq	13,059,419	2,384,245,382	365	2,384
A4.fastq	**11,122,240**	**1,918,690,099**	**363**	**1,919**
A5.fastq	9,749,376	1,752,373,416	362	1,752
A6.fastq	10,277,529	1,738,471,304	368	1,739

3. DENOISING AND CHIMERA REMOVAL:

The reads are checked for noise which refers to low confident reads. The process of removal of such reads is called as denoising. Chimeric sequences are sequences arising from PCR amplification of multiple parent fragments. Noisy and chimeric reads are removed from the downstream analysis. Statistics of the total number of denoised and the number of non-chimeric reads are given in the table below:

Table 2: Table for denoised reads and non-chimeric reads count.

Sample Name	# of Denoised Reads	# of Non-Chimeric Sequences
A1	1140718	1067857
A2	2409914	2244276
A3	2797438	2551081
A4	2383197	2051103
A5	2278880	2055543
A6	1635811	1323227

4. OTU Picking:

The OTU picking step assigns similar reads to operational taxonomic units, or OTUs, by clustering them based on a user-defined similarity threshold. Reads which are similar at or above the threshold level are taken to represent the presence of a taxonomic unit (e.g., a species, when the similarity threshold is set at 0.99) in the sequence collection. A representative sequence is chosen from each OTU.
In the next step, the frequency of occurrence of an OTU for the sample is calculated and the results are stored in a matrix where each column represents the sample identifier and each row represents the OTU and its frequency of the sample.

Table 3: OTU summary table.

OTU Summary	Count
Number of Samples	1
Number of OTUs	13,669

Table 4: OTU summary for sample.

Sample Name	A1	A2	A3	A4	A5	A6
Count	10,67,857.000	22,44,276.000	25,51,081.000	20,51,103.000	20,55,543.000	13,23,227.000

5. TAXONOMY BAR CHART PLOT:

The representative sequences for each OTU clusters are then used for taxonomic assignment. A set of representative sequences were obtained after clustering. These sequences were taken forward for the taxonomy assignment and were mapped with the 16S green genes database. The resulting taxonomy assigned OTU sequences are then used to plot the bar chart in order to depict the abundance of microorganisms at each level.

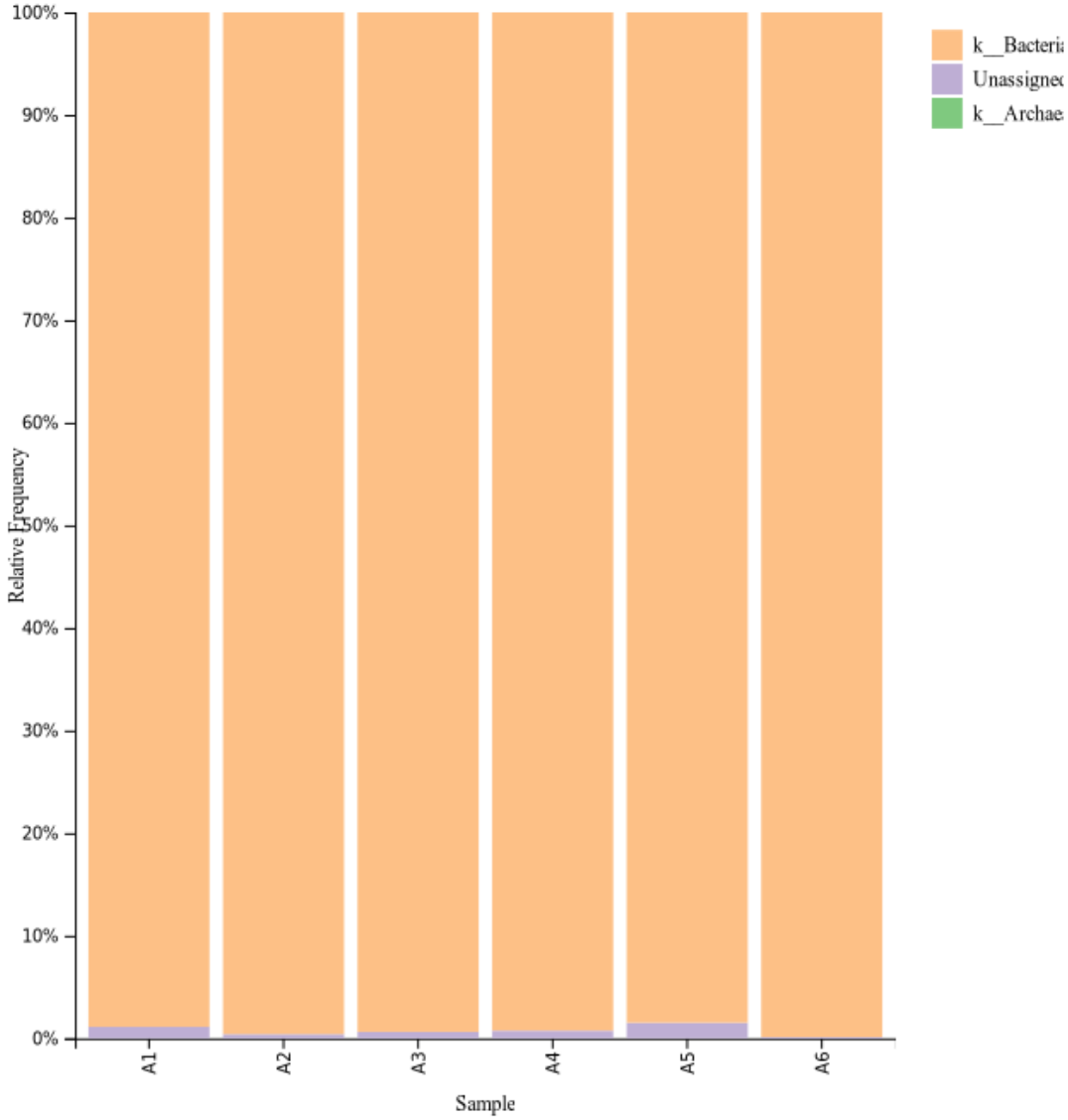

Figure 1: Kingdom level bar chart plot for sample.

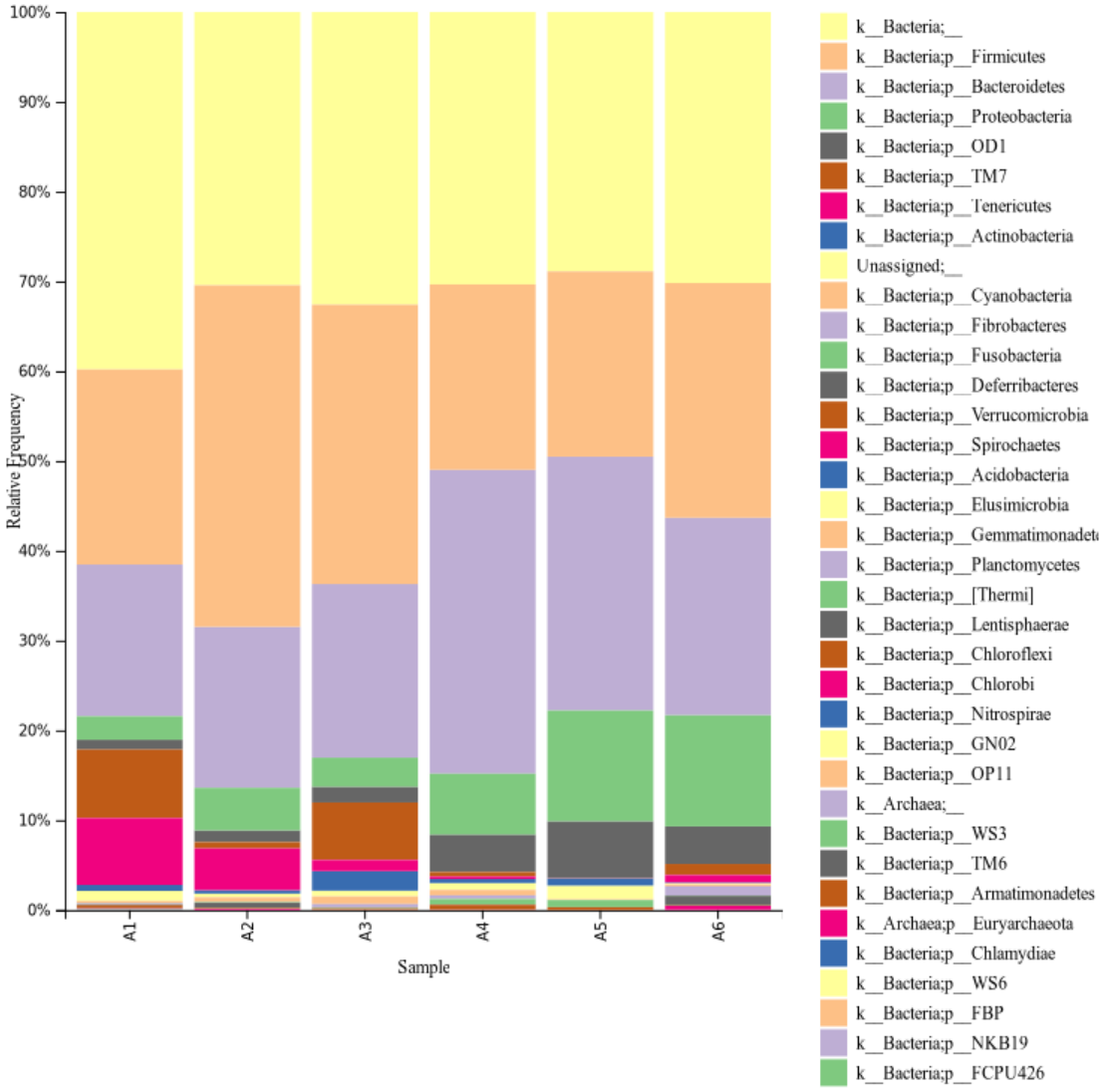

Figure 2: Phylum level bar chart plot for sample.

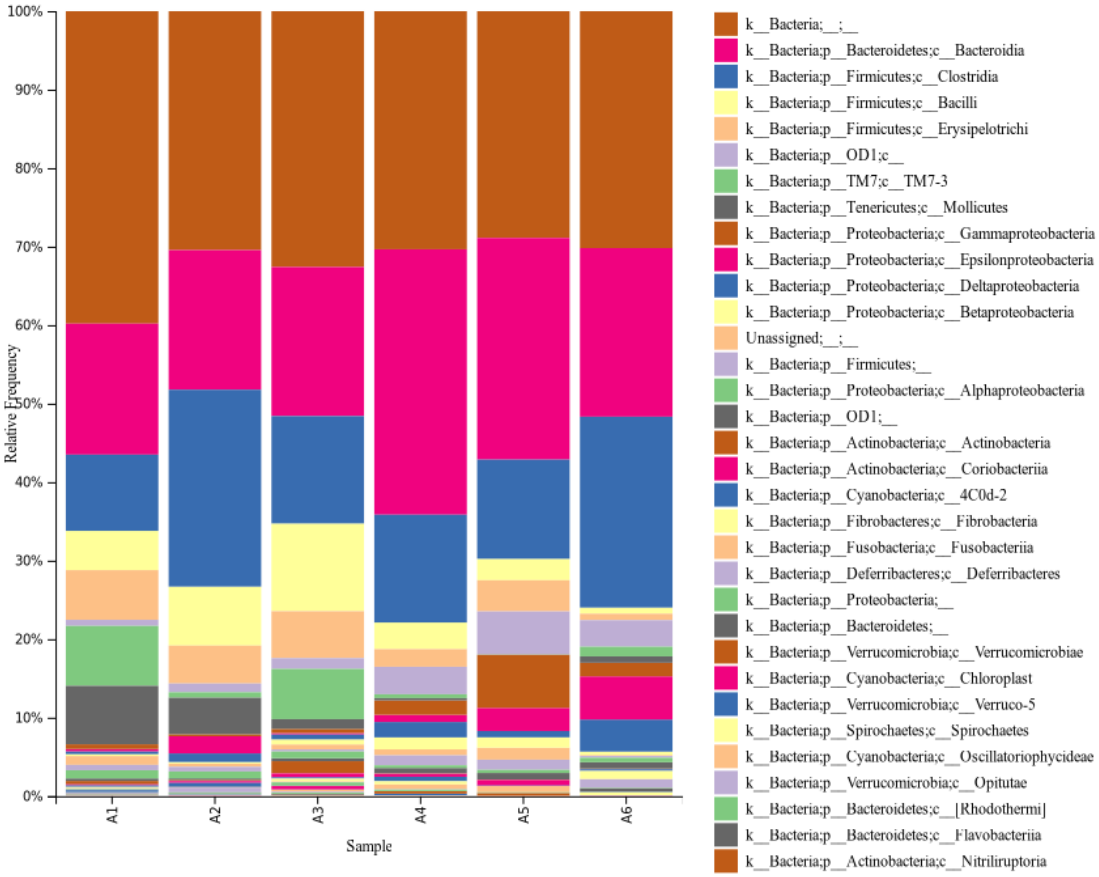

Figure 3: Class level bar chart plot for sample.

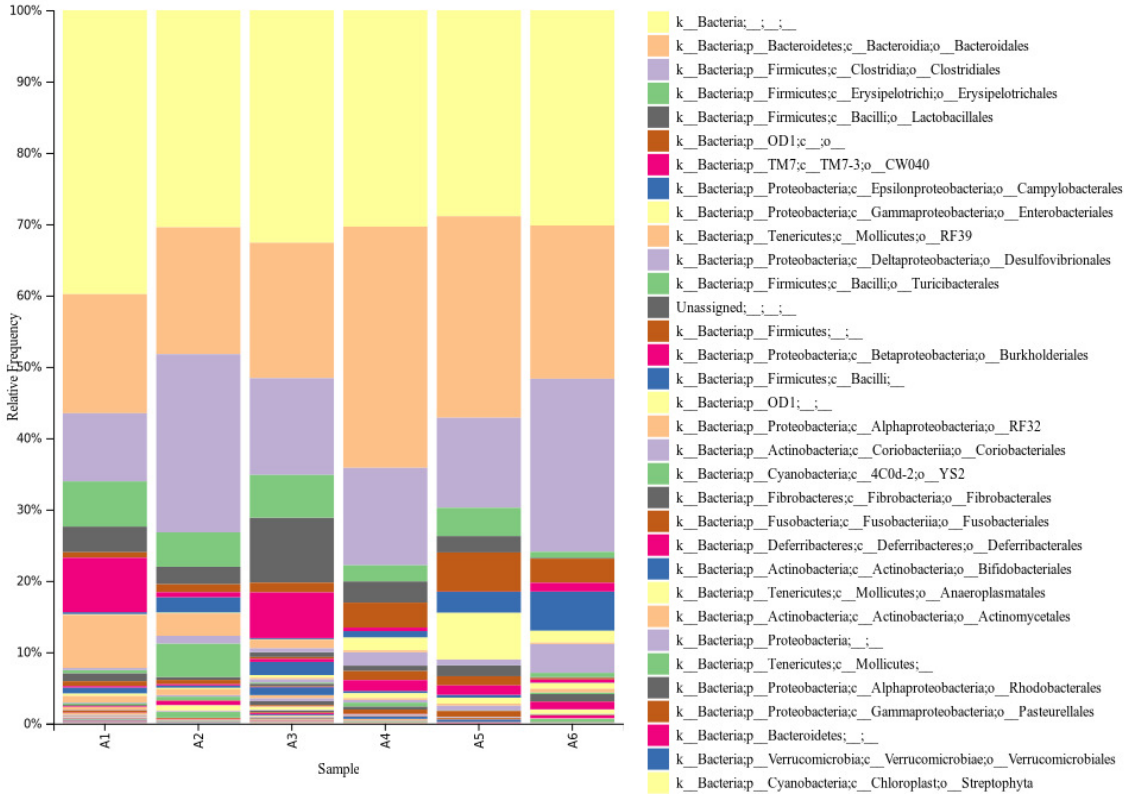

Figure 4: Order level bar chart plot for sample.

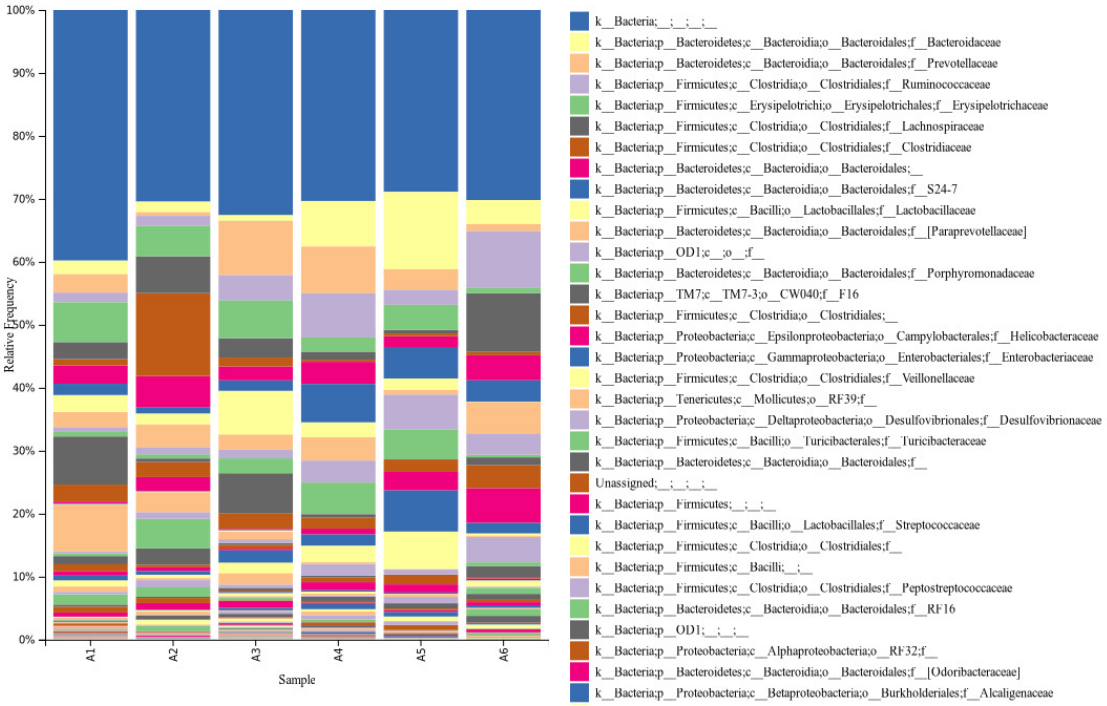

Figure 5: Family level bar chart plot for sample.

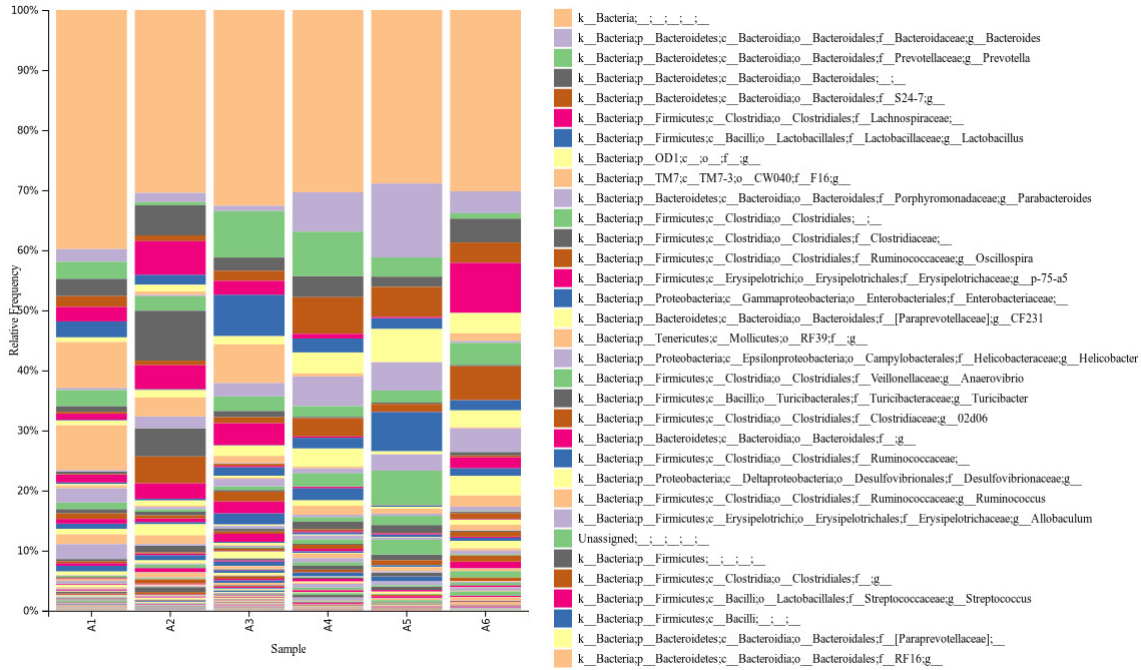

Figure 6: Genus level bar chart plot for sample.

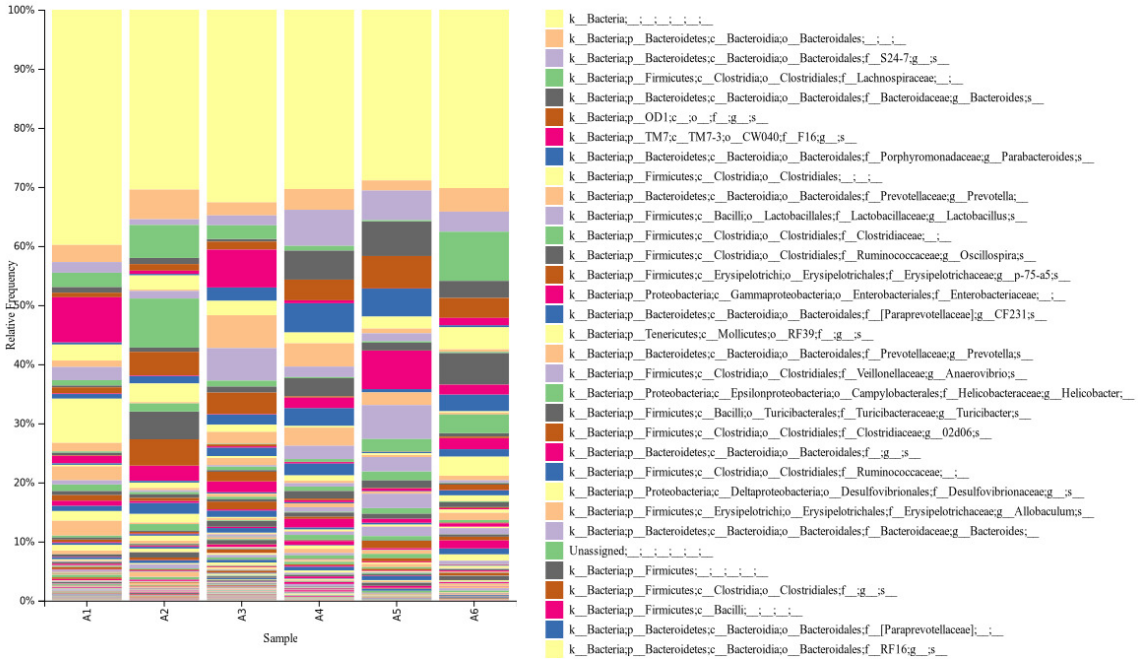

Figure 7: Species level bar chart plot for sample.

6. ALPHA DIVERSITY ANALYSIS:

Alpha diversity is a measure of diversity within a sample and indicates “richness” (number of species present in the sample) and “evenness” (evenness in the distribution of relative abundance of species).

Rarefaction plot.

The goal of rarefaction is determine whether sufficient observations have been made to get a reasonable estimate of a quantity (in this case it is the different number of species in the sample) that has been measured by sampling. Rarefaction analysis plots the count of unique OTUs (observed_species) against the number of sequences sampled. If we get a similar value of observed_species with fewer observations, then it is reasonable to infer that observed_species has converged on a good estimate of the correct value. Conversely, if observed_species is systematically increasing or decreasing as more samples are added, then we can infer that we cannot make a good estimate of observed_species for the full population.

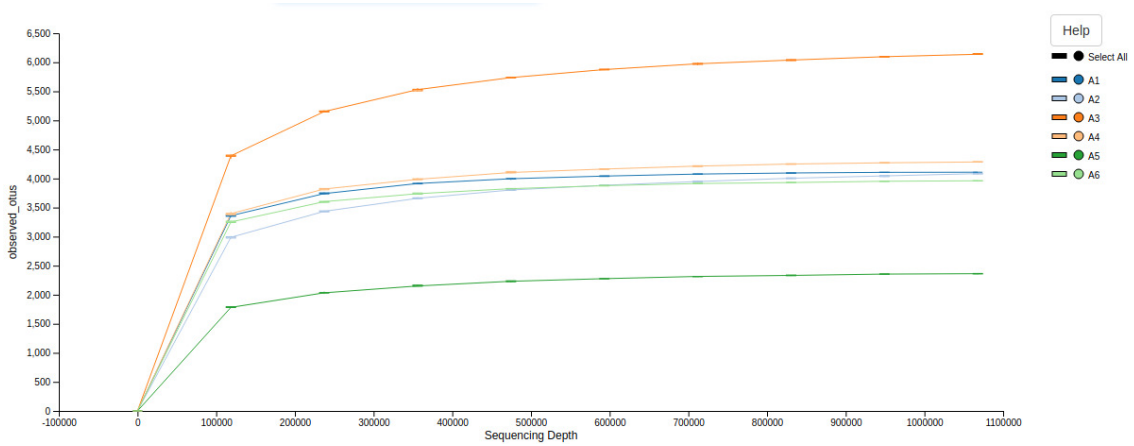

Figure 8: Rarefaction curve depicting the richness of organisms over for the sample.

[Rarefaction plot: On the X-axis, are the sequences samples in each rarefaction and on the Y -axis, the number of unique OTUs (observed_species). It is clearly evident that observed_species has converged for all samples suggesting that almost all different species which could be possibly present in the samples have been observed.]

7. Beta Diversity:

Beta diversity refers to the diversity between samples. This is essentially a measure of how similar or dissimilar the samples are, and is usually represented by a distance matrix which is then used to do Principal Coordinates Analysis (PCoA). The result of this is an ordination plot of multiple dimensions, where each sample is a point and the distance between the points represents the similarity of those samples (closer together = more similar). The similarity matrix is based on Euclidean distance between the samples as shown below:

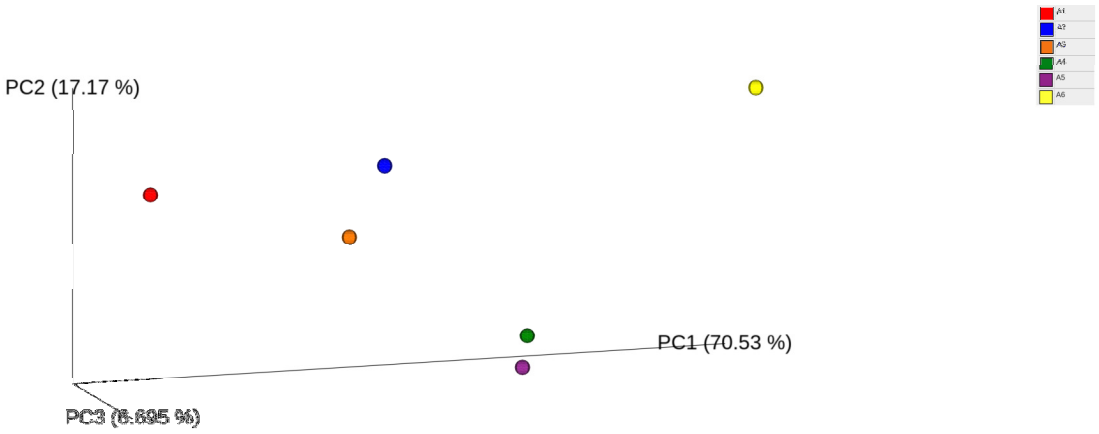

Figure 9: Beta-Diversity plot depicting the closeness of the samples with one another.

8. Heatmap:

A heatmap is generated with the annotated sequences. The heatmap represents the abundance of organism at the species level with respect to the other samples considered in the study. The left side of the heatmap is a dendrogram depicting the closeness between each annotated sequences. The right side of the heatmap image shows a bar with color range. This indicates the relative abundance of an annotated sequence within the study groups. The abundance is calculated as standard deviation of the mean value obtained for the particular annotated sequence. The light pink and red bar in the left side of the image depicts the abundance of s particular organism in all samples. The light pink denotes that there is a difference in the abundance of particular organism with the study. The red color denotes that there is a huge variation in abundance within the samples. The intermediate spaces are of white color. White color denotes that the abundance between the samples is similar. Similar abundance of species between samples shows that there is not much of a variation between the samples in terms of species. Hence the neutral white color.

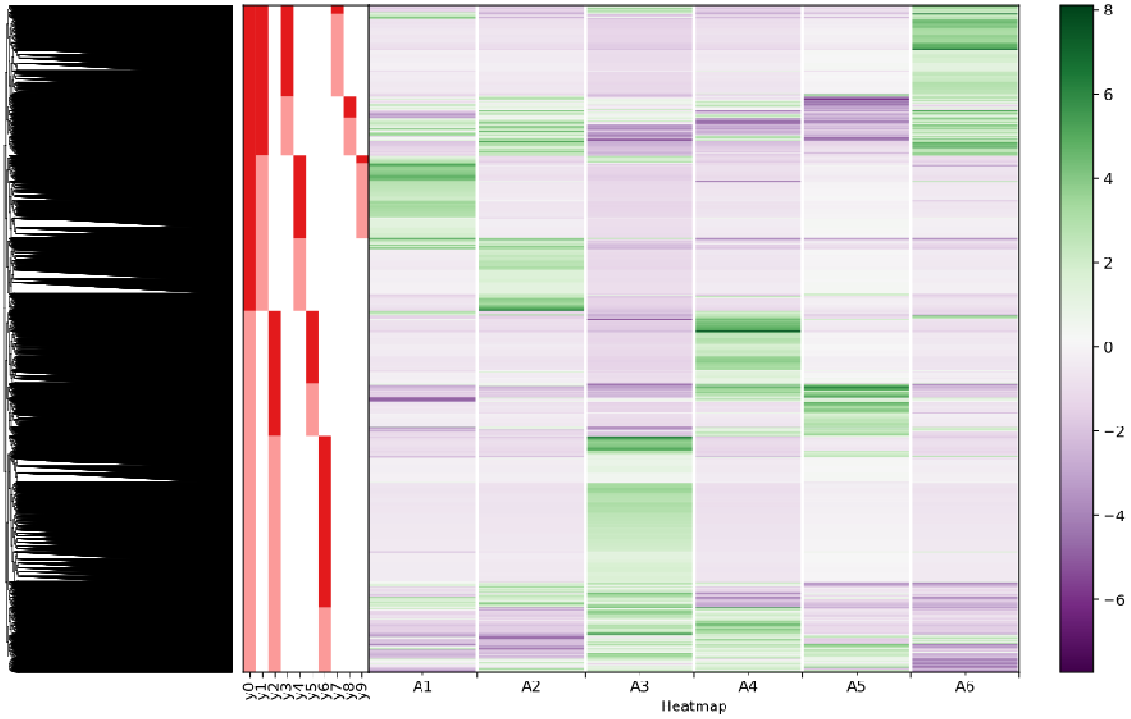

Figure 10: Heatmap of all the samples.

[END OF REPORT]