

Supporting information for:

Comparison of iSeq and MiSeq as the two platforms for 16S rRNA sequencing in the study of the gut of rats microbiome

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TABLES

Table S1: Primer sequences (Genomed, Warsaw, Poland), reaction mixtures, and thermal amplification profiles were used in the study.

Primer sequence 5'→3'	Reaction mixture	Thermal amplification profile
<u>Primers^a</u>		
F:	Molecular grade water ^b -	95 °C – 3 min
TCGTCGGCAGCGTC	10.5 µl	95 °C – 30 sec 55 °C – 30 sec 72 °C – 30 sec } 30x
AGATGTGTATAAGA	Kapa ^c - 12.5 µl	
GACAGCCTACGGGN	Pr. F (10 µM) - 0.5 µl	
GGCWGCAG	Pr. R (10 µM) - 0.5 µl	
R:		72 °C – 5 min
GTCTCGTGGGCTCG	DNA - 1.0µl	
GAGATGTGTATAAG		
AGACAGGACTACHV		
GGGTATCTAATCC		

^a reference (Klindworth et al. 2013)

^b Molecular grade water (Roche Kapa Biosystems, Wilmington, United States)

^c 2x KAPA HiFi HotStart ReadyMix (Roche Kapa Biosystems, Wilmington, United States)

Table S2: The statistically significant differences in the OTUs abundance at three taxonomic levels (family-L5, genus-L6, and species-L7).

FAMILY (L5)			
Taxonomy level	iSeq (number of classified reads)	MiSeq (number of classified reads)	p-value
<i>Bacteroidaceae</i>	1228	77	0.023
<i>Barnesiellaceae</i>	1636	7138	0.002
<i>Bifidobacteriaceae</i>	80542	44994	0.042
<i>Erysipelatoclostridiaceae</i>	1180	33	0.000
<i>Erysipelotrichaceae</i>	572102	326277	0.003
<i>Nocardiodaceae</i>	1848	157	0.001
<i>Peptostreptococcaceae</i>	2893	282221	0.000
<i>Promicromonosporaceae</i>	2	97253	0.049
<i>Tannerellaceae</i>	1903	184	0.009
<i>Thermoactinomycetaceae</i>	2637	144	0.000
GENUS (L6)			
Taxonomy level	iSeq	MiSeq	p-value
<i>Acutalibacter</i>	5165	961	0.003
<i>Adlercreutzia</i>	19961	4738	0.013
<i>Acutalibacter</i>	5165	961	0.003
<i>Adlercreutzia</i>	19961	4738	0.013
<i>Bacteroides</i>	1228	77	0.023
<i>Barnesiella</i>	1636	7138	0.002
<i>Bifidobacterium</i>	80542	44994	0.042
<i>Blautia</i>	2508	13120	0.048
<i>Cellulosimicrobium</i>	2	97253	0.049
<i>Eggerthella</i>	30783	4273	0.002
<i>Erysipelatoclostridium</i>	1180	33	0.003
<i>Faecalibaculum</i>	118755	44268	0.029
<i>Intestinimonas</i>	4561	2466	0.041
<i>Micropruina</i>	1848	157	0.001
<i>Parabacteroides</i>	1903	184	0.009

<i>Paraclostridium</i>	1426	52	0.000
<i>Paraeggerthella</i>	177	9545	0.035
<i>Pseudoflavonifractor</i>	2961	1345	0.036
<i>Romboutsia</i>	1467	282169	0.000
<i>Salinithrix</i>	2637	144	0.000
<i>Slackia</i>	655	7673	0.004
<i>Turicibacter</i>	444072	267614	0.007
SPECIES (L7)			
Taxonomy level	iSeq	MiSeq	p-value
<i>Lactobacillus gasseri</i>	0	5386600	0.000
<i>Cellulosimicrobium funkei</i>	30965	9365000	0.000
<i>Streptococcus peroris</i>	41911	229090	0.000
<i>Romboutsia ilealis</i>	75832	865970	0.000
<i>Hydrogenibacillus schlegelii</i>	0	259910	0.000
<i>Clostridium hylemonae</i>	14854	690200	0.000
<i>Rothia nasimurium</i>	19677	1457000	0.000
<i>Romboutsia timonensis</i>	66323	24694000	0.000
<i>Clostridium innocuum</i>	120390	23066	0.000
<i>Lactobacillus johnsonii</i>	8407900	496650	0.000
<i>Paraclostridium</i> <i>bifermentans</i>	140340	42066	0.000
<i>Hungateiclostridium</i> <i>cellulolyticum</i>	29631	140740	0.000
<i>Barnesiella viscericola</i>	63275	501300	0.000
<i>Paraeggerthella</i> <i>hongkongensis</i>	16673	614470	0.000
<i>Thermoactinomycetaceae</i> <i>bacterium</i>	236250	12137	0.000
<i>Rothia dentocariosa</i>	1941300	21264	0.000
<i>Micropruina glycogenica</i>	183750	20175	0.000
<i>Clostridium hiranonis</i>	132040	21916	0.000
<i>Eggerthella sinensis</i>	2930100	412140	0.000
<i>Slackia equolifaciens</i>	63522	742730	0.000

<i>Streptococcus acidominimus</i>	51355	317080	0.000
<i>Blautia producta</i>	132440	12680	0.000
<i>Clostridium quinii</i>	185810	22215	0.000
<i>Acutalibacter muris</i>	449180	114830	0.002
<i>Dorea longicatena</i>	298010	43855	0.002
<i>Christensenella timonensis</i>	214840	45360	0.003
<i>Adlercreutzia equolifaciens</i>	1827500	501180	0.005
<i>Turicibacter sanguinis</i>	43753000	27906000	0.005
<i>Parabacteroides chartae</i>	193540	27058	0.006
<i>Faecalibaculum rodentium</i>	11057000	4711000	0.013
<i>Clostridium lavalense</i>	106170	16106	0.016
<i>Barnesiella intestinihominis</i>	150120	332160	0.016
<i>Clostridium sp</i>	83249	56898	0.021
<i>Bacteroides mediterraneensis</i>	120620	87508	0.025
<i>Acetivibrio ethanolgignens</i>	30285	79817	0.033
<i>Blautia glucerasea</i>	144930	1428400	0.040
<i>Flintibacter butyricus</i>	425890	198340	0.040
<i>Bifidobacterium animalis</i>	7340000	4356800	0.045

Table S3: The features with significant differential abundance at the genus level. The statistically significant ($p < 0.05$) features are ranked by their p values, in the same samples sequenced in MiSeq and iSeq machines respectively.

Genus	p-value	MiSeq	iSeq
<i>Cellulosimicrobium</i>	0.000	936500	30
<i>Hydrogenibacillus</i>	0.000	25991	0
<i>Romboutsia</i>	0.000	2561700	15033
<i>Erysipelatoclostridium</i>	0.000	230	12039
<i>Paraclostridium</i>	0.000	420	14034
<i>Paraeggerthella</i>	0.000	61447	1667
<i>Salinithrix</i>	0.000	1213	23625
<i>Micropruina</i>	0.000	2017	18375
<i>Eggerthella</i>	0.000	41214	293010
<i>Slackia</i>	0.000	74273	6352
<i>Barnesiella</i>	0.000	83346	15645
<i>Acutalibacter</i>	0.001	11483	44918
<i>Dorea</i>	0.001	4385	29801
<i>Christensenella</i>	0.002	4536	21484
<i>Adlercreutzia</i>	0.004	50118	182750
<i>Turicibacter</i>	0.005	2790600	4375300
<i>Parabacteroides</i>	0.005	2705	19354
<i>Faecalibaculum</i>	0.012	471100.0	1105700
<i>Bacteroides</i>	0.024	875	12062
<i>Acetivibrio</i>	0.032	7981	3028
<i>Intestinimonas</i>	0.039	19834	42589
<i>Bifidobacterium</i>	0.045	435680	734000

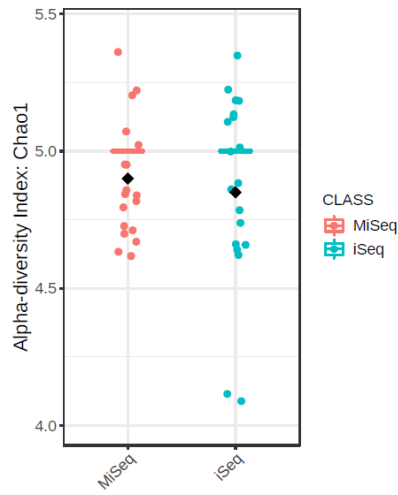
Table S4: The features with significant differential abundance at the species level. The statistically significant ($p < 0.05$) features are ranked by their p values, in the same samples sequenced in MiSeq and iSeq machines respectively.

Species	p-value	MiSeq	iSeq
<i>Lactobacillus gasseri</i>	0.000	538660	0
<i>Cellulosimicrobium funkei</i>	0.000	936500	31
<i>Streptococcus peroris</i>	0.000	22909	42
<i>Romboutsia ilealis</i>	0.000	86597	76
<i>Hydrogenibacillus schlegelii</i>	0.000	25991	0
<i>Clostridium hylemonae</i>	0.000	69020	15
<i>Rothia nasimurium</i>	0.000	145700	197
<i>Romboutsia timonensis</i>	0.000	2469400	6632
<i>Clostridium innocuum</i>	0.000	231	12039
<i>Lactobacillus johnsonii</i>	0.000	49665	840790
<i>Paraclostridium bifermentans</i>	0.000	421	14034
<i>Hungateiclostridium cellulolyticum</i>	0.000	14074	30
<i>Barnesiella viscericola</i>	0.000	50130	633
<i>Paraeggerthella hongkongensis</i>	0.000	61447	1667
<i>Thermoactinomycetaceae bacterium</i>	0.000	1214	23625
<i>Rothia dentocariosa</i>	0.000	213	194130
<i>Micropruina glycogenica</i>	0.000	2018	18375
<i>Clostridium hiranonis</i>	0.000	2192	13204
<i>Eggerthella sinensis</i>	0.000	41214	293010
<i>Slackia equolifaciens</i>	0.000	74273	6352
<i>Streptococcus acidominimus</i>	0.000	31708	5136
<i>Blautia producta</i>	0.000	1268	13244
<i>Clostridium quinii</i>	0.000	2222	18581
<i>Acutalibacter muris</i>	0.001	11483	44918
<i>Dorea longicatena</i>	0.001	4386	29801
<i>Christensenella timonensis</i>	0.002	4536	21484

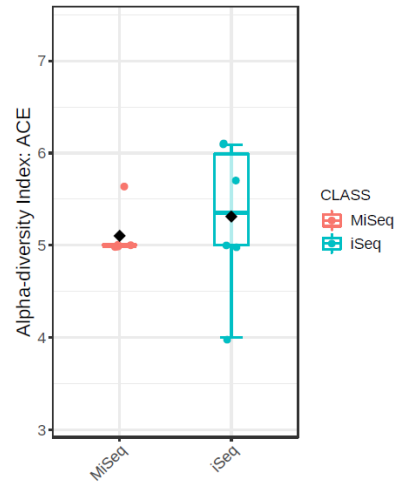
<i>Adlercreutzia equolifaciens</i>	0.004	50118	182750
<i>Turicibacter sanguinis</i>	0.005	2790600	4375300
<i>Parabacteroides chartae</i>	0.005	2706	19354
<i>Faecalibaculum rodentium</i>	0.012	471100	1105700
<i>Clostridium lavalense</i>	0.015	1611	10617
<i>Barnesiella intestinihominis</i>	0.016	33216	15012
<i>Clostridium sp</i>	0.021	5690	8325
<i>Bacteroides mediterraneensis</i>	0.024	875	12062
<i>Acetivibrio ethanoligignens</i>	0.032	7982	3029
<i>Blautia glucerasea</i>	0.039	142840	14493
<i>Flintibacter butyricus</i>	0.039	19834	42589
<i>Bifidobacterium animalis</i>	0.045	435680	734000

FIGURES

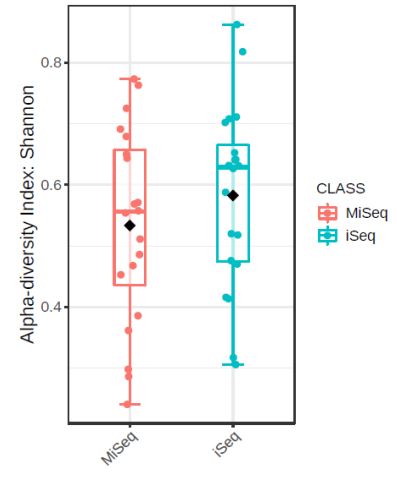
Chao1: p -value: 0.64302



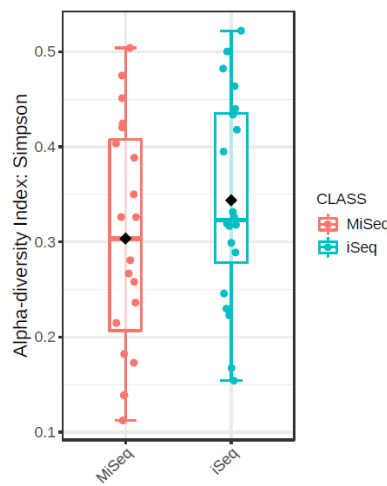
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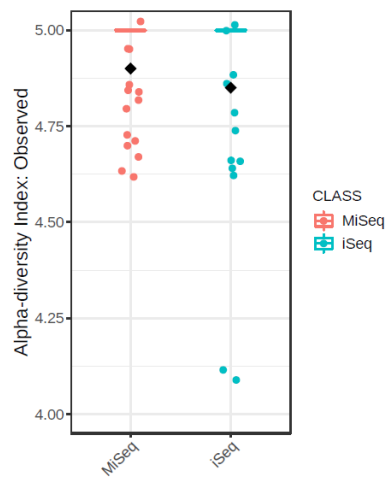
Shannon: p -value: 0.32535



Simpson: p -value: 0.27783



Observed: p -value: 0.643



Fisher: p -value: 0.195

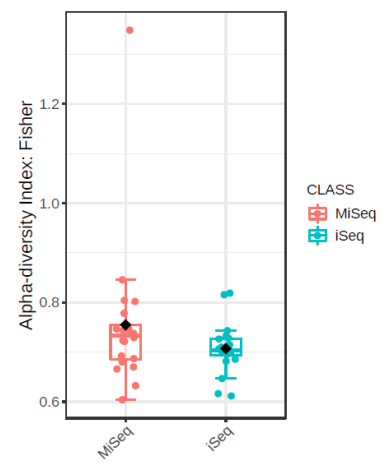
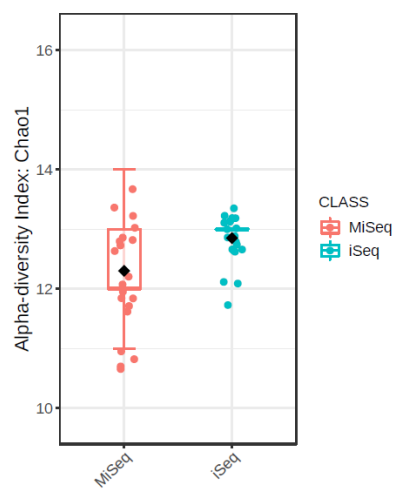
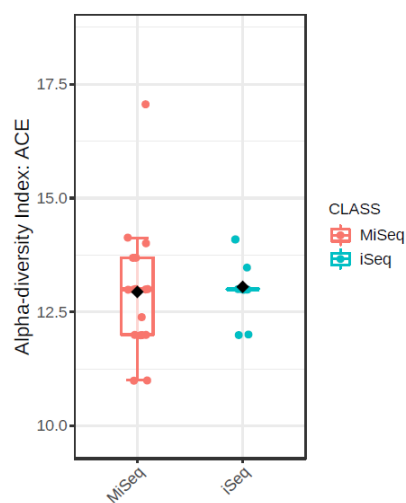


Figure S1: Alfa diversity expressed in six indices at the phylum and class levels.

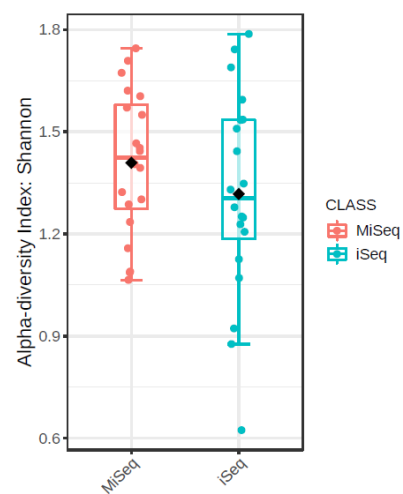
Chao1: p -value: 0.014592



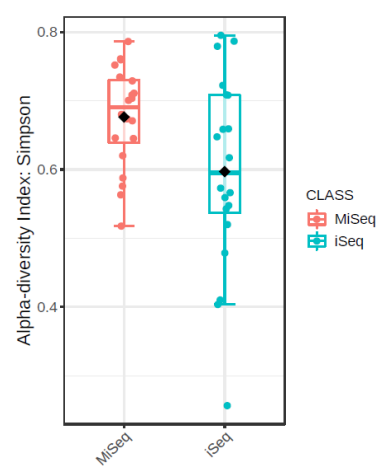
ACE: p -value: 0.77503



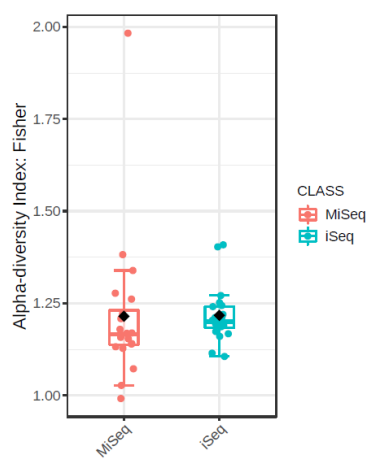
Shannon: p -value: 0.27357



Simpson: p -value: 0.033438



Fisher: p -value: 0.959



Observed: p -value: 0.003

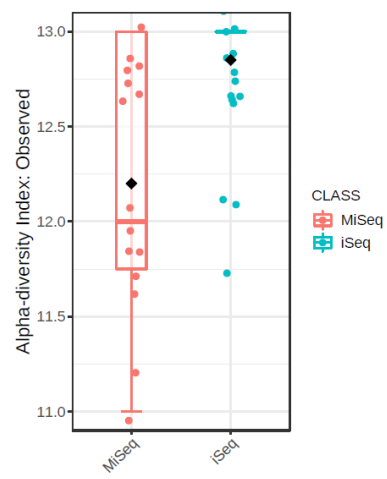
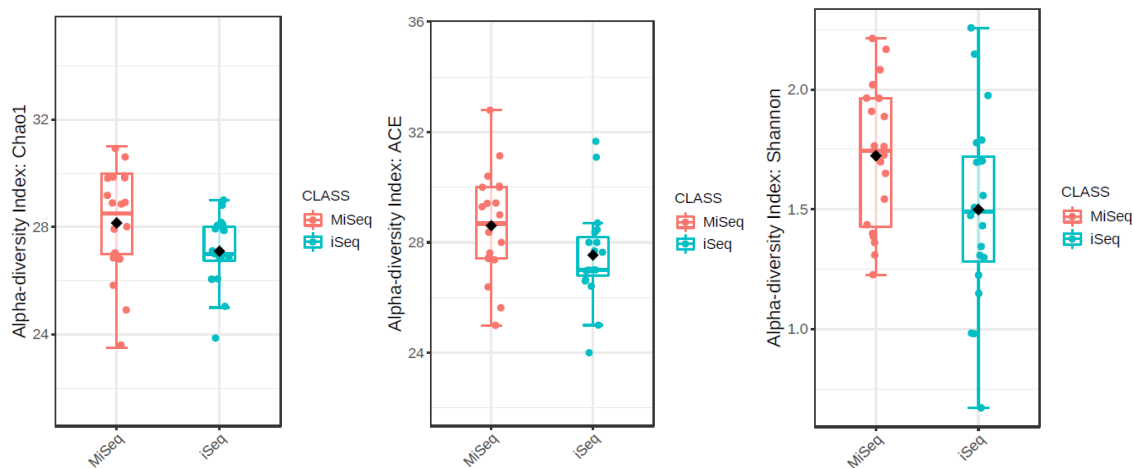


Figure S2: Alfa diversity expressed in six indices at the order level.

Chao1: p -value: 0.051934

ACE: p -value: 0.077

Shannon: p -value: 0.052



Simpson: p -value: 0.004

Observed: p -value: 0.032

Fisher: p -value: 0.015

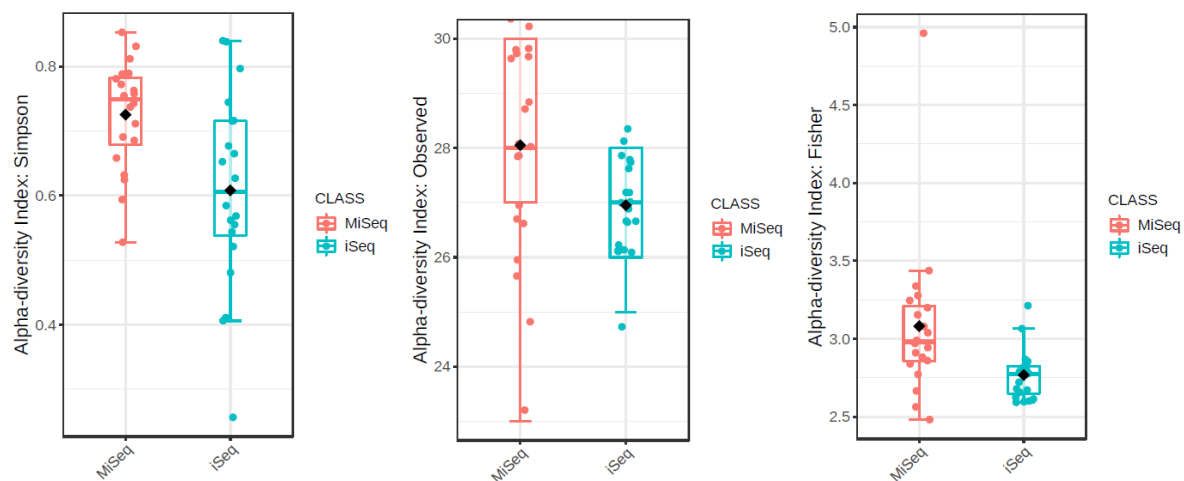
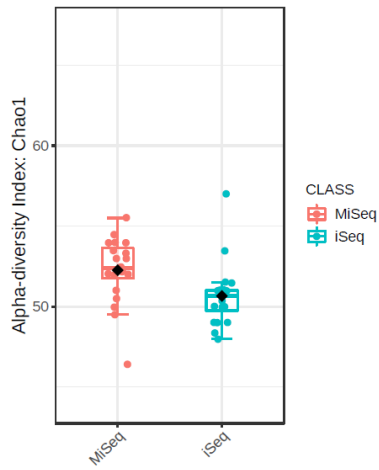
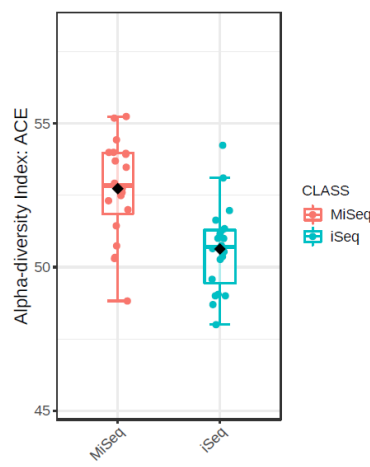


Figure S3: Alfa diversity expressed in six indices at the family level.

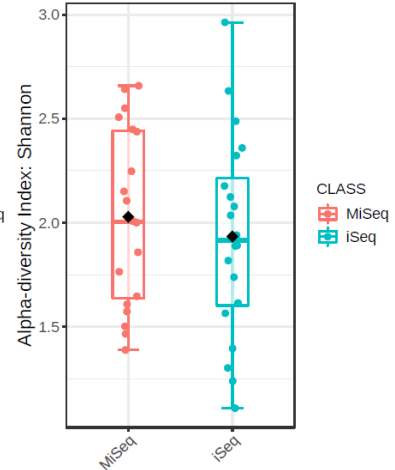
Chao1: p -value: 0.016



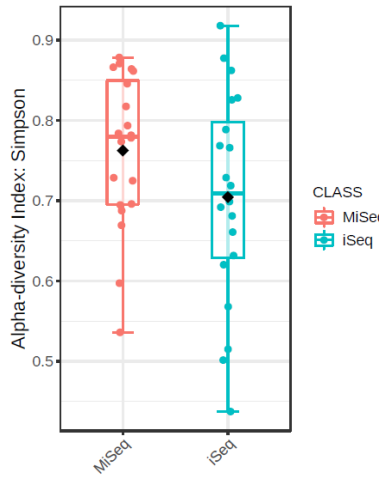
ACE: p -value: 0.000



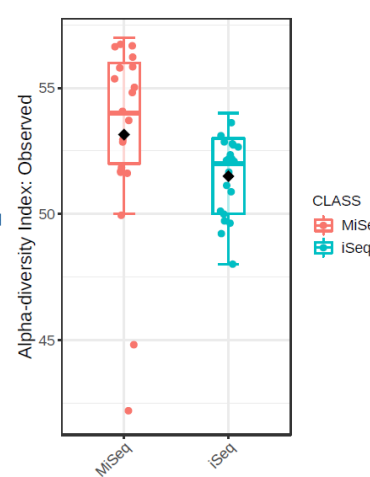
Shannon: p -value: 0.514



Simpson: p -value: 0.118



Observed: p -value 0.090



Fisher, p -value: 0.024

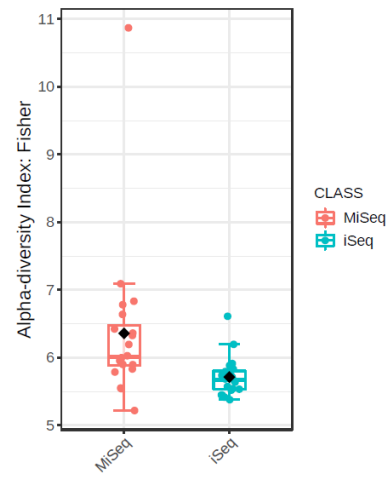
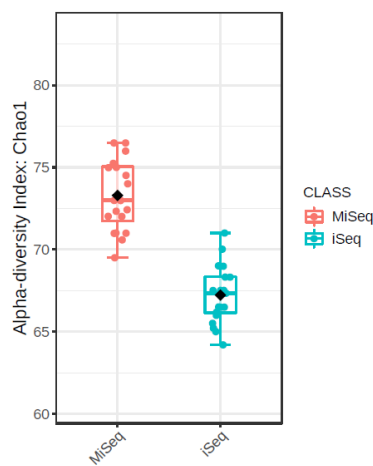
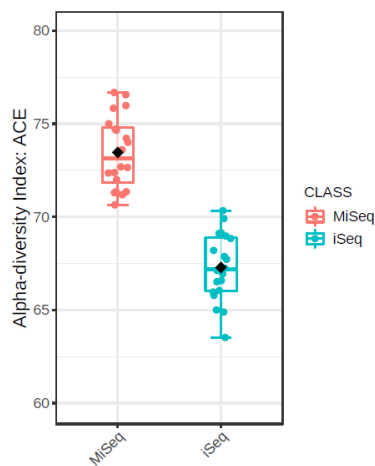


Figure S4: Alfa diversity expressed in six indices at the genus level.

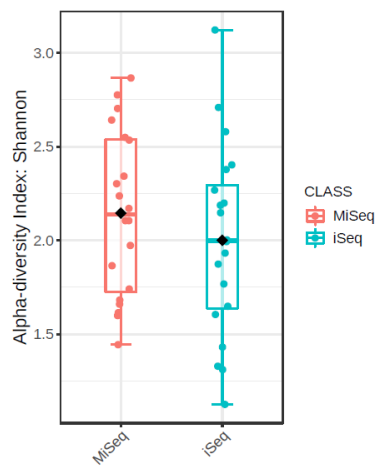
Chao1: p -value: 0.000



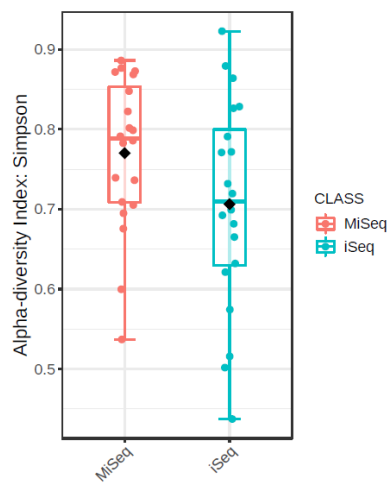
ACE: p -value: 0.000



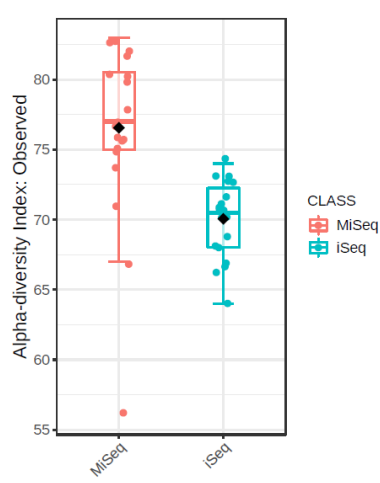
Shannon: p -value: 0.341



Simpson: p -value: 0.088



Observed: p -value < 0.000



Fisher: p -value: 0.000

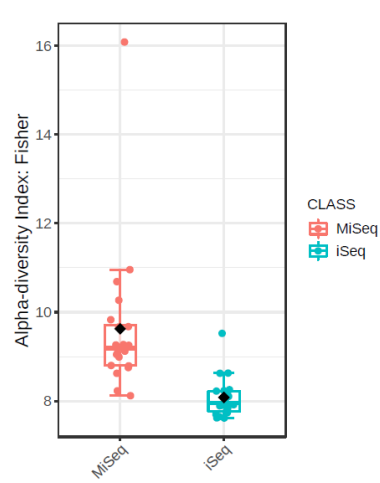


Figure S5: Alfa diversity expressed in six indices at the species level.

[PERMANOVA] F-value: 0.11248; R-squared: 0.0029513; p -value < 0.82

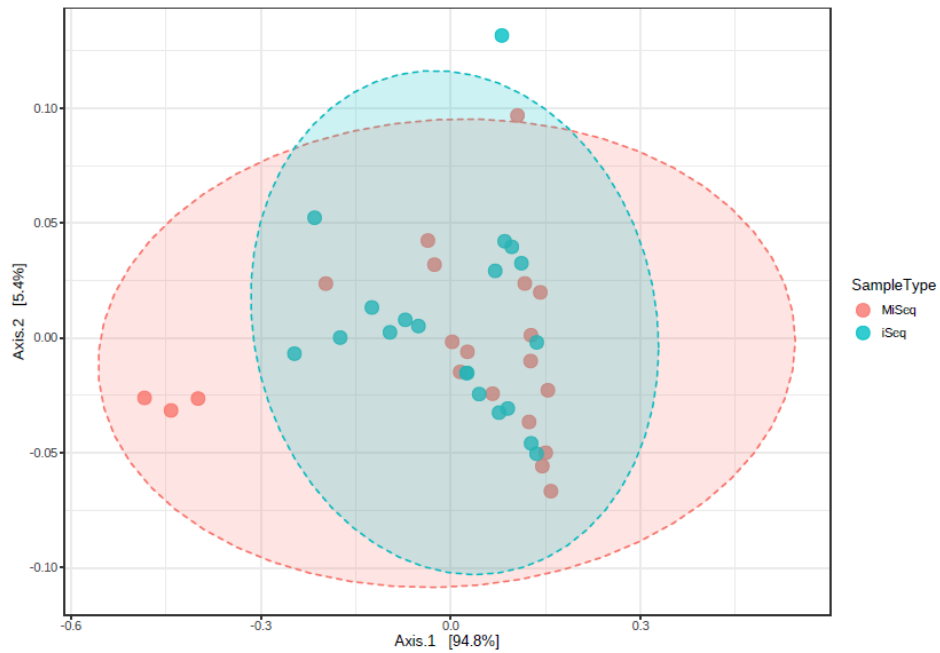


Figure S6: Beta diversity expressed with the Bray-Curtis index at the phylum level, visualized by principal coordinate analysis (PCoA).

[PERMANOVA] F-value: 15.589; R-squared: 0.2909; p -value < 0.001

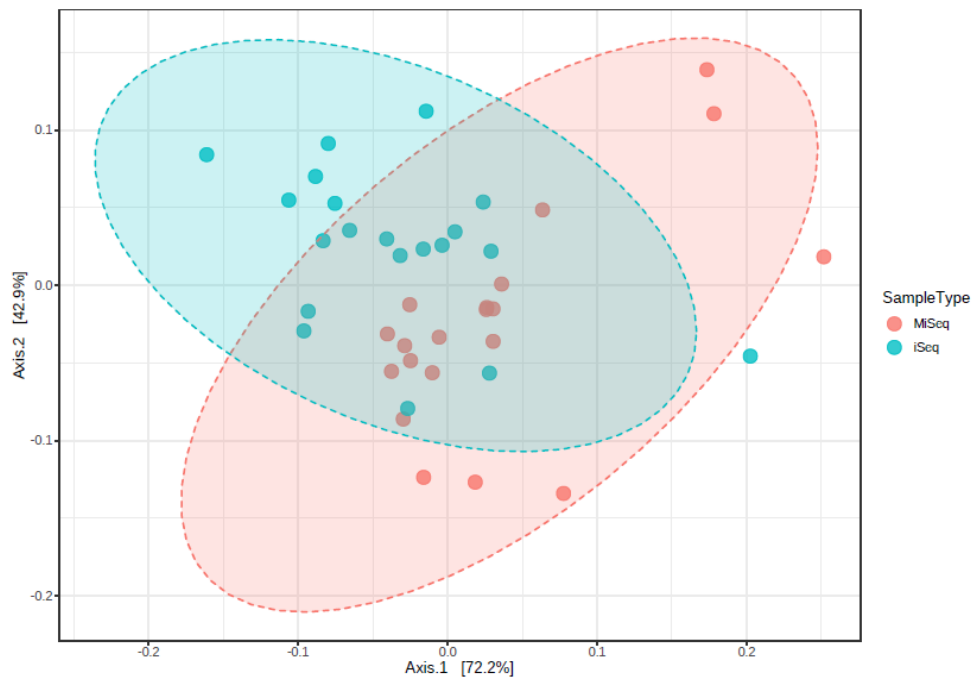


Figure S7: Beta diversity expressed with the Bray-Curtis index at the class level, visualized by principal coordinate analysis (PCoA).

[PERMANOVA] F-value: 16.27; R-squared: 0.29979; p -value < 0.001

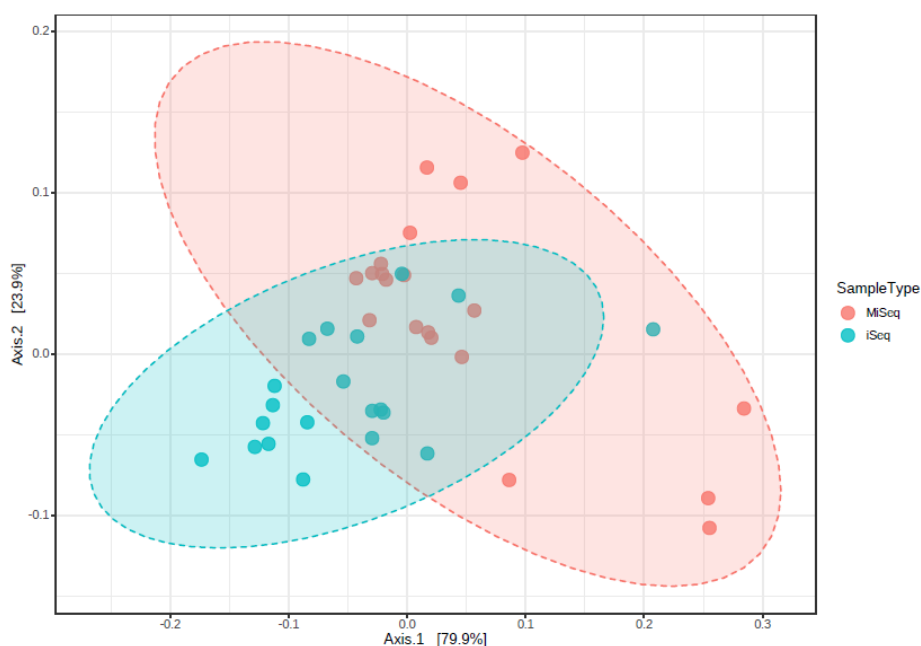


Figure S8: Beta diversity expressed with the Bray-Curtis index at the order level, visualized by principal coordinate analysis (PCoA). A significant correspondence between beta-diversity estimates (MiSeq - red separate from iSeq – blue) were demonstrated.

[PERMANOVA] F-value: 24.182; R-squared: 0.38889; p -value < 0.001

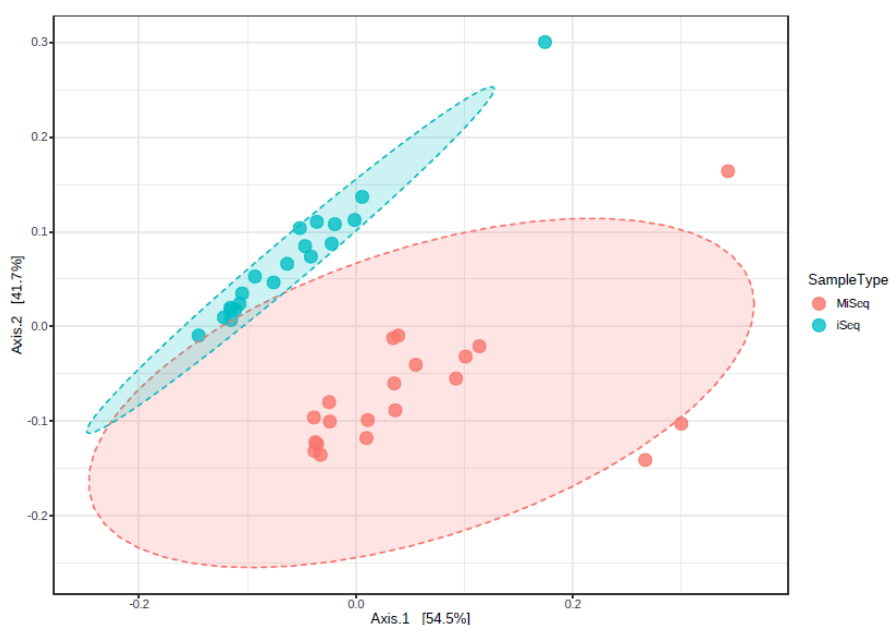


Figure S9: Beta diversity expressed with the Bray-Curtis index at the family level, visualized by principal coordinate analysis (PCoA). A significant correspondence between beta-diversity estimates (MiSeq - red separate from iSeq – blue) were demonstrated.

[PERMANOVA] F-value: 21.899; R-squared: 0.3656; p -value < 0.001

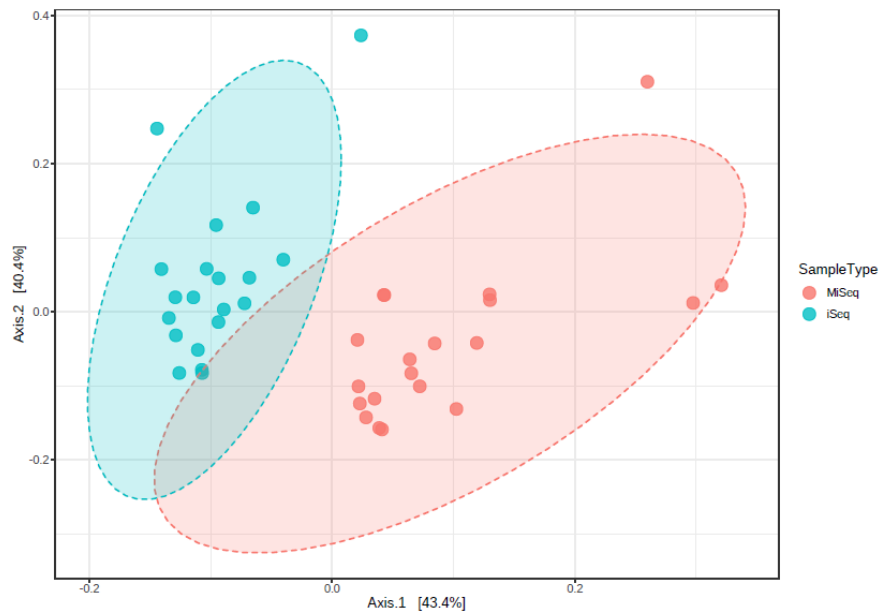


Figure S10: Beta diversity expressed with the Bray-Curtis index at the genus level, visualized by principal coordinate analysis (PCoA). A significant correspondence between beta-diversity estimates (MiSeq - red separate from iSeq – blue) were demonstrated.

[PERMANOVA] F-value: 31.408; R-squared: 0.45251; p -value < 0.001

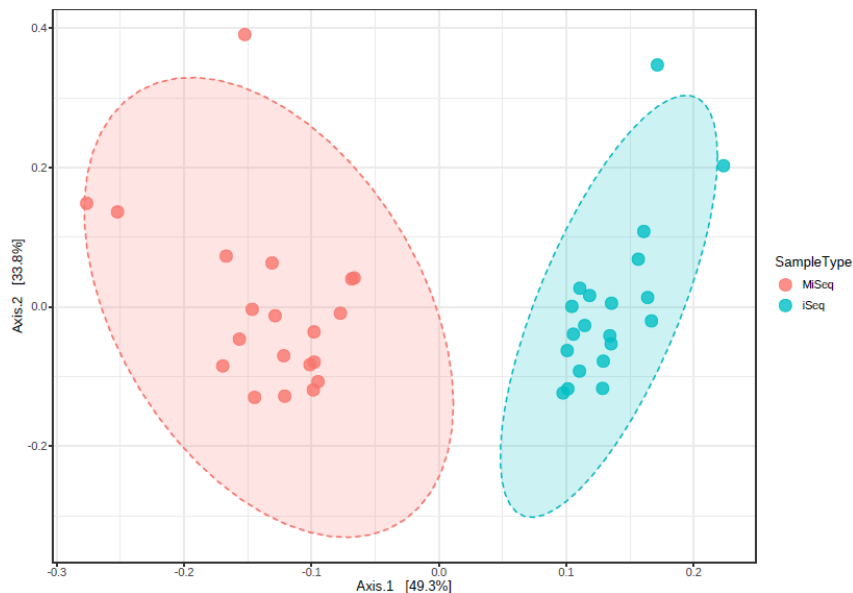


Figure S11: Beta diversity expressed with the Bray-Curtis index at the species level, visualized by principal coordinate analysis (PCoA). A significant correspondence between beta-diversity estimates (MiSeq - red separate from iSeq – blue) were demonstrated.