

Tendentious effects of automated and manual metagenomic DNA purification protocols on broiler gut microbiome taxonomic profiling

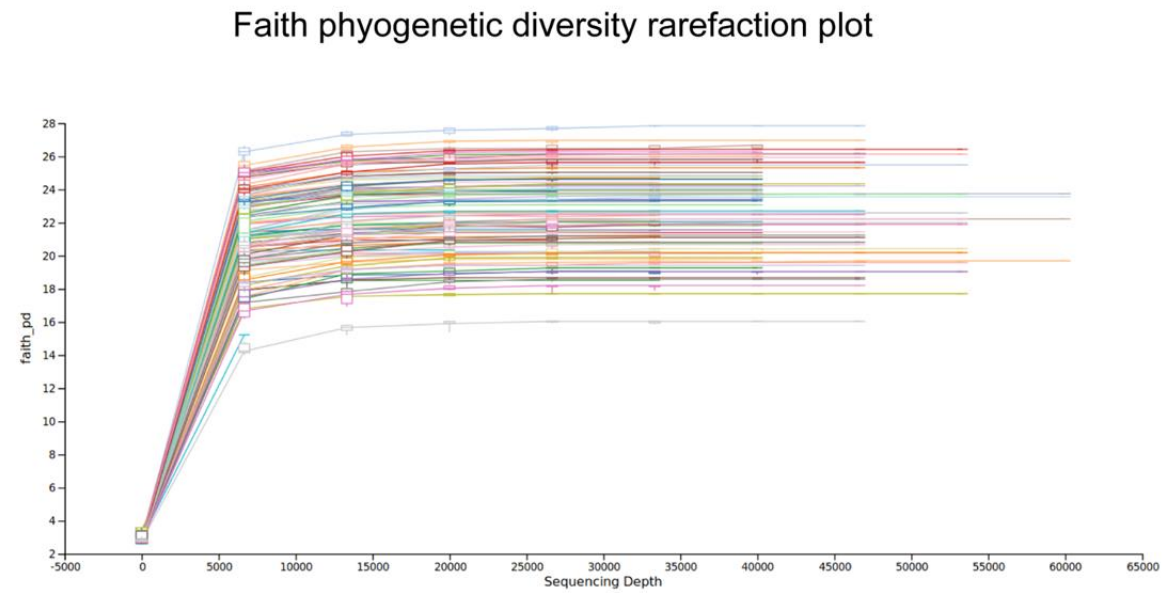
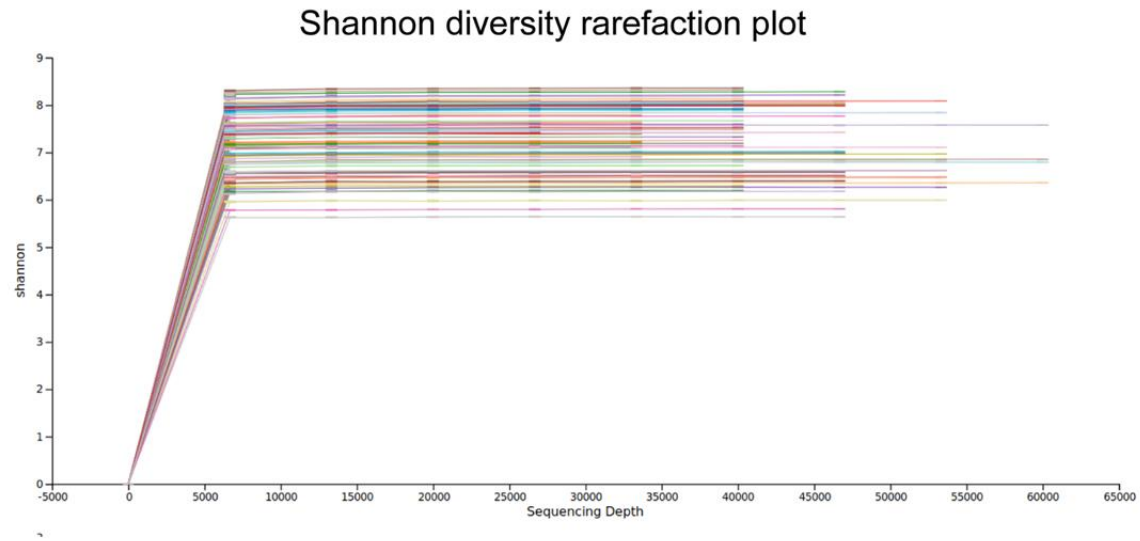
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	A	B	C	D	E	F	G	H
1	BS 1.1 4.1	BS 1.5 4.2	BS 1.6+2.1 4.1	RS 2.2 4.2	BS 2.2 4.2	BS 1.2 6	BS 2.2 1.3 6	RS 2.2 6
2	BS 1.2 4.1	BS 2.2 4.2	BS 2.2 1.2 4.1	RS 2.2 4.2	BS 2.5 5.1	BS 1.3 6	BS 1.1 2.2 6	RS 2.3 6
3	BS 1.3 4.1	BS 2.5 4.2	BS 2.2 1.4 4.1	RS 2.3 4.2	RS 2.5 5.1	BS 1.4 6	BS 1.3 2.2 6	RS 2.4 6
4	BS 1.4 4.1	BS 2.2 4.2	BS 2.2 4.1	RS 2.1 1.4 4.1	BS 2.1 1.1 5.2	BS 2.5 6	BS 1.1+2.1 6	RS 2.1 1.1 6
5	BS 1.5 4.1	BS 2.1 1.2 4.1	BS 2.4 4.1	RS 1.2 2.1 4.1	BS 2.1 1.2 5.2	BS 2.2 6	BS 1.3+2.1 6	RS 2.1 1.3 6
6	BS 1.6 4.1	BS 2.1 1.4 4.1	RS 2.1 1.2 4.2	RS 1.4 2.1 4.1	RS 2.1 1.1 5.2	BS 2.3 6	BS 1.1+2.2 6	RS 1.1 2.1 6
7	BS 2.1 4.1	BS 2.1 1.6 4.1	RS 2.1 1.4 4.2	RS 1.2+2.1 4.1	RS 2.1 1.2 5.2	BS 2.4 6	BS 1.3+2.2 6	RS 1.1 2.1 6
8	BS 2.2 4.1	BS 1.2 2.1 4.1	RS 1.2 2.1 4.2	RS 1.4 2.1 4.1	BS 2.1 1.1 5.3	BS 2.1 1.1 6	RS 1.1 6	RS 2.2 1.1 6
9	BS 1.1 4.2	BS 1.4 2.1 4.1	RS 1.4 2.1 4.2	RS 2.2 4.1	BS 2.1 1.2 5.3	BS 2.1 1.3 6	RS 1.2 6	RS 2.2 1.3 6
10	BS 1.2 4.2	BS 1.6 2.1 4.1	RS 1.2+2.1 4.1	RS 2.2 4.1	RS 2.1 1.1 5.3	BS 1.1 2.1 6	RS 1.3 6	RS 1.1 2.2 6
11	BS 1.3 4.2	BS 1.2+2.1 4.1	RS 1.4+2.1 4.1	RS 2.4 4.1	RS 2.1 1.2 5.3	BS 1.3 2.1 6	RS 1.4 6	RS 1.3 2.2 6
12	BS 1.4 4.2	BS 1.4+2.1 4.1	RS 2.2 1.2 4.2	BS 2.1 4.2	BS 1.1 6	BS 2.2 1.1 6	RS 2.1 6	RS 1.1+2.1 6

Supplementary Figure S1. Experimental design indicating protocol manipulations of the 96 methods.



Supplementary Figure S2. Sequencing read depth in correlation with alpha-diversity. Plots show, that 5000 reads per sample are enough to maximize the Shannon's and Faith phylogenetic diversity. Increasing the read number will not affect the overall diversity significantly, but can cause the loss of samples.

Stirring (RS)							
S1(P4.1)	S2 (P4.2)	S3 (P5.1)	S4 (P5.2)	S5 (P5.3)	S6 (P6-L1)	S7 (P6-L2)	S8 (P6-L3)
Roche MagNa Pure 24 Pathogen 200	Roche MagNa Pure 24 Pathogen 1000	QIAmp DNA Stool Mini kit	QIAmp Power Fecal kit	DNeasy Power Soil kit	Conventional		
D11, D10, D9	D2, D1, C12	E3	E6, E7	E10, E11	G8, G9, G10, G11	G12, H1, H2, H3	H4, H5, H6, H7, H8, H9, H10, H11, H12
Washing (BS)							
S9(P4.1)	S10 (P4.2)	S11 (P5.1)	S12 (P5.2)	S13 (P5.3)	S14 (P6-L1)	S15 (P6-L2)	S16 (P6-L3)
Roche MagNa Pure 24 Pathogen 200	Roche MagNa Pure 24 Pathogen 1000	QIAmp DNA Stool Mini kit	QIAmp Power Fecal kit	DNeasy Power Soil kit	Conventional DNA precipitation		
A8, C5, C4	B4, E1, B2	E2	E4, E5	E8, E9	E12, F1, F2, F3	F4, F5, F6, F7	F8, F9, F10, F11, F12, G1, G2, G3, G4, G5, G6, G7

Supplementary Table S1. Representation of plate positions associated with standard metagenome DNA isolation methods.

	Ranking of methods according to:		
Sample Id	(C) Shannon's Diversity Index	(B) Observed OTUs	(A) DNA Concentration ng/ μ L
A1	64	76	67
A2	65	82	51
A3	38	39	75
A4	45	42	71
A5	57	59	86
A6	56	41	84
A7	44	1	50
A8	84	68	70
A9	47	55	38
A10	50	64	26
A11	35	45	24
A12	70	70	48
B1	37	13	33
B2	69	74	82
B3	31	31	9
B4	34	15	13
B5	26	40	37

B6	30	46	35
B7	16	11	36
B8	21	28	15
B9	5	7	29
B10	17	19	19
B11	13	12	40
B12	28	36	44
C1	3	2	61
C2	4	5	58
C3	6	6	60
C4	2	3	59
C5	1	4	76
C6	66	38	62
C7	53	9	53
C8	58	47	52
C9	54	35	46
C10	61	51	56
C11	51	50	45
C12	41	54	80
D1	59	58	72
D2	39	72	74
D3	49	80	78

D4	60	67	68
D5	40	52	63
D6	62	69	49
D7	55	65	73
D8	52	83	66
D9	42	62	77
D10	7	48	81
D11	46	49	79
D12	48	73	30
E1	43	37	64
E2	80	84	85
E3	83	81	89
E4	81	85	93
E5	79	63	90
E6	90	91	92
E7	94	93	88
E8	68	60	87
E9	76	57	95
E10	73	78	91
E11	82	79	94
E12	32	34	96
F1	33	44	39

F2	24	29	41
F3	74	56	18
F4	9	8	83
F5	10	10	42
F6	12	23	20
F7	14	18	16
F8	18	22	31
F9	20	32	22
F10	25	16	12
F11	19	20	11
F12	29	14	21
G1	23	25	27
G2	8	21	43
G3	15	24	32
G4	67	26	69
G5	11	27	17
G6	22	33	10
G7	36	87	34
G8	85	30	25
G9	89	88	28
G10	72	86	57
G11	88	89	4

G12	96	96	54
H1	92	94	23
H2	91	90	2
H3	93	92	3
H4	86	75	5
H5	75	43	65
H6	95	95	47
H7	63	53	8
H8	71	61	6
H9	77	66	14
H10	78	77	7
H11	27	17	1
H12	87	71	55

Supplementary Table S2. Rankings of the 96 different metagenomic DNA extractions to observed ASV numbers (column B) in relation of DNA yield (column A) and Shannon's diversity indexes (column C).

Bacterial Suspension (BS)												
Phylum		Class		Order		Family		Genus		Genus		Species
<i>Actinobacteria</i>	1.1020979882	<i>Clostridia</i>	32.100664176	<i>Clostridiales</i>	32.08896673	<i>Ruminococcaceae</i>	19.5283391	<i>Lactobacillus</i>	18.9450832	<i>Unknown</i>		86.88301289
<i>Bacteroidetes</i>	28.688718888	<i>Bacteroidia</i>	28.686742912	<i>Bacteroidales</i>	28.62921766	<i>Lactobacillaceae</i>	18.9450832	<i>Bacteroides</i>	14.1091148	<i>Lactobacillus salivarius</i>		2.677829001
<i>Cyanobacteria</i>	0.6418643311	<i>Bacilli</i>	25.548865867	<i>Lactobacillales</i>	25.18859237	<i>Bacteroidaceae</i>	14.1091148	<i>Unknown</i>	9.52379486	<i>Bacteroides gallinaceum</i>		2.617802658
<i>Epsilonbacteraeota</i>	1.9119322117	<i>Gammaproteobacteria</i>	5.7824991659	<i>Enterobacteriales</i>	4.643268449	<i>Lachnospiraceae</i>	7.42733638	<i>Alistipes</i>	6.25301334	<i>Lactobacillus aviarius</i>		2.202312630
<i>Firmicutes</i>	59.941162436	<i>Campylobacteria</i>	1.9118005247	<i>Campylobacterales</i>	1.911800524	<i>Rikenellaceae</i>	6.25316725	<i>Streptococcus</i>	4.98307141	<i>Bacteroides uniformis</i>		1.50146660
<i>Lentisphaerae</i>	0.0048870965	<i>Erysipelotrichia</i>	1.7904010952	<i>Erysipelotrichales</i>	1.790401095	<i>Streptococcaceae</i>	4.99869365	<i>Escherichia-Shigella</i>	4.62510663	<i>Bacteroides sp. SB5</i>		0.80546886
<i>Patescibacteria</i>	0.00019240537	<i>Verrucomicrobiae</i>	0.9621145279	<i>Verrucomicrobiales</i>	0.962114527	<i>Enterobacteriaceae</i>	4.64326844	<i>Ruminococcaceae UCG-014</i>	4.16882947	<i>Anaeromassilibacillus senegalensis</i>		0.66279086
<i>Proteobacteria</i>	6.57260609232	<i>Actinobacteria</i>	0.7717232831	<i>Gastranaerophilales</i>	0.631623339	<i>Barnesiellaceae</i>	3.30529667	<i>Faecalibacterium</i>	3.78900894	<i>Campylobacter jejuni</i>		0.638434020
<i>Tenericutes</i>	0.17435775085	<i>Melainabacteria</i>	0.6316233393	<i>Pseudomonadales</i>	0.629122241	<i>Unknown</i>	3.09835970	<i>Barnesiella</i>	2.41613723	<i>Lactobacillus vaginalis</i>		0.214978958
<i>Verrucomicrobia</i>	0.96218079948	<i>Negativicutes</i>	0.4939475362	<i>Corynebacteriales</i>	0.575829623	<i>Prevotellaceae</i>	2.18026449	<i>Prevotellaceae UCG-001</i>	2.17964883	<i>Mordavella massiliensis</i>		0.16003
Raw Stool Specimens (RS)												
Phylum		Class		Order		Family		Genus		Genus		Species
<i>Actinobacteria</i>	1.3776821185	<i>Bacilli</i>	56.004035651	<i>Lactobacillales</i>	55.86466177	<i>Lactobacillaceae</i>	52.0731793	<i>Lactobacillus</i>	52.0731793	<i>Unknown</i>		89.3344130
<i>Bacteroidetes</i>	12.627088818	<i>Clostridia</i>	23.436784551	<i>Clostridiales</i>	23.43405173	<i>Ruminococcaceae</i>	11.4854888	<i>Unknown</i>	5.80891780	<i>Lactobacillus salivarius</i>		3.21000502
<i>Cyanobacteria</i>	0.5814475562	<i>Bacteroidia</i>	12.626525430	<i>Bacteroidales</i>	12.42518902	<i>Lachnospiraceae</i>	6.41248084	<i>Bacteroides</i>	5.64176955	<i>Lactobacillus aviarius</i>		2.20901172
<i>Epsilonbacteraeota</i>	0.5006863033	<i>Gammaproteobacteria</i>	2.4160368629	<i>Erysipelotrichales</i>	1.603775531	<i>Bacteroidaceae</i>	5.64176955	<i>Barnesiella</i>	3.61200857	<i>Lactobacillus vaginalis</i>		1.68531398
<i>Firmicutes</i>	81.435174314	<i>Erysipelotrichia</i>	1.6037755316	<i>Enterobacteriales</i>	1.349623177	<i>Barnesiellaceae</i>	3.84374064	<i>Streptococcus</i>	3.51223272	<i>Phaseolus acutifolius</i>		0.43412813
<i>Fusobacteria</i>	0.0011154869	<i>Actinobacteria</i>	1.2297694558	<i>Pseudomonadales</i>	0.722580184	<i>Streptococcaceae</i>	3.51245580	<i>Romboutsia</i>	3.21876120	<i>Bacteroides sp. SB5</i>		0.39084917
<i>Lentisphaerae</i>	0.0007250664	<i>Campylobacteria</i>	0.5006639639	<i>Micrococcales</i>	0.643161059	<i>Peptostreptococcaceae</i>	3.38306509	<i>Faecalibacterium</i>	2.89338819	<i>Lactobacillus oris</i>		0.26764914
<i>Patescibacteria</i>	0.0002788717	<i>Oxyphotobacteria</i>	0.4671450776	<i>Campylobacterales</i>	0.500663963	<i>Unknown</i>	2.11146674	<i>Ruminococcaceae UCG-014</i>	1.85848560	<i>Bacteroides gallinaceum</i>		0.21159842
<i>Proteobacteria</i>	3.06876028	<i>Negativicutes</i>	0.3863316562	<i>Chloroplast</i>	0.467145077	<i>Erysipelotrichaceae</i>	1.60377553	<i>[Ruminococcus] torques</i>	1.64577174	<i>Lactobacillus pontis</i>		0.19152055
<i>Tenericutes</i>	0.217966143	<i>Deltaproteobacteria</i>	0.3846585005	<i>Corynebacteriales</i>	0.463017960	<i>Christensenellaceae</i>	1.35726392	<i>Subdoligranulum</i>	1.42117847	<i>Lactobacillus panis</i>		0.17863725

Supplementary Table S3. Silva databases taxonomic data. Relative frequencies are shown for the ten most abundant taxa in Phylum, Class, Order, Family, Genus and Species level.

Silva databases								
<i>Family (L5)</i>			<i>Genus (L6)</i>			<i>Species (L7)</i>		
Group	Identified	Not identified	Group	Identified	Not identified	Group	Identified	Not identified
BS	99.49	0.51	BS	96.69	3.31	BS	14.42	85.58
RF	98.13	1.87	RF	97.41	2.59	RF	9.16	90.84
L1	99.71	0.29	L1	95.11	4.89	L1	10.71	89.29
L2	97.85	2.15	L2	96.41	3.59	L2	11.48	88.52
L3	99.50	0.50	L3	98.27	1.73	L3	16.72	83.28
P4	99.25	0.75	P4	94.98	5.02	P4	15.09	84.91
P5	95.04	4.96	P5	94.79	5.21	P5	7.16	92.84
P6	94.65	5.35	P6	94.23	5.77	P6	18.28	81.72
mean	97.95	2.05	mean	95.99	4.01	mean	12.88	87.12
SD	2.03	2.03	SD	1.42	1.42	SD	3.86	3.86
Greengenes databases								
<i>Family (L5)</i>			<i>Genus (L6)</i>			<i>Species (L7)</i>		
Group	Identified	Not identified	Group	Identified	Not identified	Group	Identified	Not identified
BS	88.81	11.19	BS	74.81	25.19	BS	44.49	55.51
RF	92.16	7.84	RF	81.99	18.01	RF	56.15	43.85
L1	85.00	15.00	L1	74.35	25.65	L1	50.63	49.37
L2	91.50	8.50	L2	78.77	21.23	L2	51.33	48.67
L3	91.63	8.37	L3	78.65	21.35	L3	48.08	51.92
P4	87.64	12.36	P4	70.31	29.69	P4	41.81	58.19
P5	93.04	6.96	P5	88.59	11.41	P5	68.68	31.32
P6	92.48	7.52	P6	83.46	16.54	P6	52.71	47.29
mean	90.28	9.72	mean	78.86	21.14	mean	51.74	48.26
SD	2.84	2.84	SD	5.80	5.80	SD	8.22	8.22

Supplementary Table S4. Table shows the percentages of the identified reads at certain taxonomic levels bound to protocol manipulations.