SUPPLEMENTAL MATERIALS

Supplemental Methods

Plasmid standard curves (Calculating the $\log_{10}$ 16S rRNA gene copies)

1. *Calculating the total number of plasmid copies (i.e., 16S rRNA gene copies because there is one copy of this gene per plasmid) from purified plasmid DNA material utilizes the following formulae:*

$$\mathbf{X} = \frac{\mathbf{m}*\mathbf{A}}{\mathbf{p}*\mathbf{Z}*\mathbf{Y}}, \text{ where:}$$

m = purified plasmid DNA concentration in ng/µL considering that 1 µL of the plasmid working solution is used per qPCR reaction
A= Avogadro's number ($6.02*10^{23}$/mole)
p = plasmid DNA size in base pairs (bp), which equals the plasmid insert size (3900 bp) + the 16S rRNA gene fragment length ranging between 1400-1600 bp
Z = the average molecular weight of a double-stranded DNA molecule, which equals 650 g/mole
Y = 10^9 to convert the value of Z from grams to nanograms, since m is in nanograms (i.e., 1 gram = 10^9 nanograms)

2. *Transforming X to the estimated total number of bacteria based on 16S rRNA copy number:*

$$\mathbf{G} = \frac{\mathbf{X}}{\mathbf{C}}, \text{ where:}$$

X = total number of plasmid copies
C = 5, which refers to the estimated number of 16S rRNA gene copies per ASF bacterial genome or a single cell (this number can be adjusted by users to whatever value they choose; it can also be a different number for each ASF member if desired)

3. *Log_{10} transformation to achieve the final value for the log_{10} total 16S rRNA gene copies contained in the purified plasmid DNA:*

$$\mathbf{F_{x,g}} = \mathbf{log_{10}}\ (\mathbf{G}), \text{ where:}$$

G = estimated total log10 of total gene (16S rRNA) copies (i.e., total bacterial abundance)

Example of a calculation using the steps described above considering the following values for *Clostridium sp.* (ASF 356):

m = 7.63 ng/µL
A = $6.02*10^{23}$/mole
p = (3900 + 1487) bp = 5387 bp
Z = 650 g/mole
Y = 10^9

$$\mathbf{X = \frac{7.63*6.02*10^{23}}{5387*650*10^{9}},}$$

$$\mathbf{X = 1.31*10^{9},}$$

$$\mathbf{G = \frac{1.31*10^{9}}{5},}$$

$$\mathbf{G = 2.62*10^{8},}$$

$$\mathbf{F_{x,g} = \log_{10}(2.62 * 10^{8}),}$$

$F_{x,g}$ = 8.4 (final value used in the linear regression model to construct the qPCR standard curve)

Genomic DNA standard curves (Calculating the $\log_{10}$ of total genome copies)

1. *Derivation of DNA mass formulae:*

$$\mathbf{m} = (\mathbf{n}) * \left(\frac{\mathbf{1\ mole}}{\mathbf{6.02 * 10^{23}}}\right) * \left(\frac{\mathbf{650\ grams}}{\mathbf{mole}}\right),$$

$$\mathbf{m} = (\mathbf{n}) * \left(\mathbf{1.08} * \frac{\mathbf{10^{-21} grams}}{\mathbf{bp}}\right), \text{where:}$$

m = genome mass
n = DNA size (in bp), which is the full genome length per ASF taxon (see Table S3).
Avogadro's number = $6.02*10^{23}$ molecules/mole
Average molecular weight of a double-stranded DNA molecule = 650 grams/mole

After m is calculated, its value is transformed from grams to femtograms (i.e., 1 gram = 10^{15} femtograms) as follows:

$$\mathbf{m1} = (\mathbf{n}) * \left(\mathbf{1.08} * \frac{\mathbf{10^{-21} grams}}{\mathbf{bp}} * \mathbf{10^{15}}\right),$$

$$\mathbf{m2} = (\mathbf{n}) * \left(\mathbf{1.08} * \frac{\mathbf{10^{-6} femtograms}}{\mathbf{bp}}\right)$$

After quantification of the purified genomic DNA material extracted from 1 mL of pure culture of the ASF taxon, its concentration value (ng/μL) is then transformed to femtograms (i.e., 1 nanogram = 10^6 femtograms) to calculate how many genome copies are present in the extract. For that, the following calculation is performed:

$$\mathbf{Final\ genome\ copies} = \frac{\mathbf{[DNA\ ng/\mu L] * 10^6\ (transforming\ to\ femtograms)}}{\mathbf{m2\ (as\ calculated\ above)}}$$

For example:

If a purified genomic DNA sample from 1 mL of a *Clostridium sp.* (ASF 356) pure culture gave a concentration of 2.9 ng/μL, then the formulae derived above would be applied as follows:

$$\mathbf{m2} = (\mathbf{2,900,700\ bp\ for\ the\ full\ genome\ length}) * \left(\mathbf{1.08} * \frac{\mathbf{10^{-6} femtograms}}{\mathbf{bp}}\right),$$

$$\mathbf{m2 = 3.13\ \frac{femtograms}{genome}\ which\ is\ the\ genome\ mass\ for\ ASF\ 356}$$

$$\textbf{Final genome copies} = \frac{[\textbf{2.9 ng/}\mu\textbf{L}] * \textbf{10}^{\textbf{6}}\ (\textbf{transforming to femtograms})}{\textbf{3.13}\ (\textbf{as calculated above})},$$

$$\textbf{Final genome copies} = \textbf{9.26} * \textbf{10}^{\textbf{5}}\textbf{genome copies}$$

*Of note, the final genome copy number does not correct for the number of 16S rRNA copies for this organism (reported in this paper as 5 per ASF taxon). The plasmid and genomic DNA initial working concentrations used to construct the qPCR standard curves are shown in Table S4.

Calculation example (Log_{10} of total 16S rRNA gene copies [i.e., estimated bacterial abundance] for ASF 356 in cecal contents from C3H/HeN mice fed standard mouse chow)

The calculation example below shows exactly how the Ct values generated during the qPCR assay (i.e., in duplicate) using SYBR Green Master Mix A were used to calculate the final $\log_{10}$ of total 16S rRNA copy number (i.e., estimated total bacterial abundance) per gram of cecal sample for one C3H/HeN mouse. This calculation was similarly used for all other ASF taxa (and for samples tested with Mix B) to determine the final population abundances in fecal or cecal samples from both host genotypes and across all studies.

1. *Calculating the average of Ct values from a duplicate run using our qPCR method:*

$$\boldsymbol{Ct_x = \frac{Ct\ value\ 1 + Ct\ value\ 2}{2}}, where:$$

Ct = cycle threshold value ranging from 0 to 35

2. *Calculating the log_{10} of total 16S rRNA copy number using the predictive equation derived from the Mix A plasmid standard curves (Table S4):*

For this example, the calculation will be based on the predictive equation generated for *L. murinus* (ASF 361):

Standard curve equation: $\boldsymbol{Y = -3.952 * X + 45.82}, where:$

Y = average Ct value calculated in step 1 (Ct_x)
X = $\log_{10}$ of total 16S rRNA gene copies

Given the values of the Ct values 1 (16.70) and 2 (17.00) achieved for a particular sample for ASF 361, total gene copies are calculated as follows:

$$\boldsymbol{Ct_x = \frac{16.70 + 17}{2} = 16.85}, \text{then:}$$

$$\boldsymbol{16.85 = -3.952 * X + 45.82,} \text{ then:}$$

$$\boldsymbol{X = 7.34\ (log_{10} of\ total\ 16S\ rRNA\ gene\ copies)}$$

3. *Back transforming the value achieved in step 2 from log_{10} to exponential:*

$$\boldsymbol{\sigma = POWER(10, X)}, \text{where:}$$

X = the value calculated in step 2 for the $\log_{10}$ of total 16S rRNA gene copies

$$\boldsymbol{\sigma = POWER(10, 7.34) = 2.17 * 10^7 total\ 16S\ rRNA\ gene\ copies}$$

4. *Calculating the total DNA amount in the eluted sample after extraction:*

$$\boldsymbol{\rho = 100\ \mu L\ *\left[DNA\frac{ng}{\mu L}\right]}, \text{where:}$$

Elution volume using TE buffer = 100 µL
[DNA ng/µL] = DNA concentration (as determined using a fluorescent molecule labeling method, i.e., Quant-iT™ PicoGreen® dsDNA Broad Range Reagent)

For this example, the values are:

$$\boldsymbol{\rho = 100\ \mu L\ *\left[174\frac{ng}{\mu L}\right]}, \text{then:}$$

$$\boldsymbol{\rho = 17400\ ng\ is\ total\ amount\ of\ DNA\ in\ the\ extracted\ sample}$$

5. *Define the amount of DNA template used in the final qPCR reaction:*

The amount of DNA loaded per reaction = 10 ng
*All samples are diluted to 10 ng/µL prior to running the qPCR, and then 1 µL of the diluted sample is used in the reaction.

6. *Calculating the normalizing factor (i.e., scaling value) used to determine the final amount of 16S rRNA gene copy number in the extracted material:*

$$\boldsymbol{\tau = \frac{\rho}{10\ ng}}, \text{where:}$$

ρ = the total amount of DNA in the sample calculated in step 4
10 ng = the amount of DNA loaded in the qPCR reaction using 1 µL of the diluted sample

For the sample using the ASF 361 calculation example, the value is as follows:

$$\boldsymbol{\tau = \frac{17400}{10\ ng} = 1740}$$

7. *Multiplying the value calculated in step 6 by the total number of bacteria or 16S rRNA copies calculated in step 3:*

$$\boldsymbol{Total\ 16S\ rRNA\ copy\ number\ in\ the\ sample\ accounting\ for\ the\ DNA\ amount = \sigma * \tau}$$

For this example, the calculation is:

$$\boldsymbol{Total\ 16S\ rRNA\ copy\ number\ in\ the\ sample\ accounting\ for\ the\ DNA\ amount = 2.17 * 10^7 * 1740}, \text{then:}$$

$$\boldsymbol{Total\ 16S\ rRNA\ copy\ number\ in\ the\ sample\ accounting\ for\ the\ DNA\ amount = 3.78 * 10^{10}}$$

8. *Dividing the value calculated in step 7 by the cecal content weight (i.e., wet value) in grams to get the estimated total bacteria/gram of cecal content (using the same example from above):*

$$\frac{\boldsymbol{Total\ 16S\ rRNA\ copy\ number}}{\boldsymbol{grams\ of\ cecal\ content}} = \frac{\boldsymbol{3.78 * 10^{10}}}{\boldsymbol{0.224\ grams}} = \boldsymbol{1.69 * 10^{11}}$$

9. *Back transforming the value calculated in step 8 from exponential to log_{10} to achieve the final total number of log10 of 16S rRNA copy number/grams of cecal content:*

$$\frac{\boldsymbol{Total\ log_{10}16S\ rRNA\ copy\ number}}{\boldsymbol{grams\ of\ cecal\ content}} = \boldsymbol{log_{10}(1.69 * 10^{11}) = 11.23}$$

Table S1a. Composition of the standard chow diet (LabDiet® JL Rat and Mouse/Auto 6F 5K67, LabDiet). Complete details can be found at http://www.labdiet.com/Products/StandardDiets.

	LabDiet 5K67
Nutrients and Minerals	**% of ration**
Protein	19.3
Fat (ether extract)	6.2
Fat (acid hydrolysis)	7.2
Fiber (crude)	4.3
Nitrogen-free extract (by difference)	53.6
Ash	6.5
Total Digestible Nutrients, %	**76.3**
Metabolizable Energy, kcal/gm	**3.17**
Calories provided by	**%**
Protein	22.24
Fat (ether extract)	16.03
Carbohydrate	61.73

Table S1b. Composition of the purified experimental diets produced by Research Diets, Inc. (New Brunswick, NJ, USA). Mice were fed either a low-fat diet (LFD, D12450K) or a customized Western diet (WD, 45% kcal from fat and 17% kcal from sucrose with low maltodextrine/high starch compared to D12451). Complete diet formulations can be accessed at (http://www.researchdiets.com/opensource-diets/stock-diets/dio-series-diets).

	Low-fat Diet (D12450K)	**Western Diet (modified D12451)**
	g (%)	**g (%)**
Protein	19.2	23.7
Carbohydrate	67.3	46.1
Fat	4.3	23.6
	kcal (%)	**kcal (%)**
Protein	20	20
Carbohydrate	70	34.1
Fat	10	44.9
Ingredient quantity	**g**	**g**
Casein, 30 Mesh	200	200
L-cystine	3	3
Corn Starch	550	137.3
Maltodextrine 10	150	35.5
Sucrose	0	172.8
Cellulose BW200	50	50
Soybean Oil	25	25
Lard	20	177.5
Mineral Mix S10026	10	10
DiCalcium Phosphate	13	13
Calcium Carbonate	5.5	5.5
Potassium Citrate, 1H2O	16.5	16.5
Vitamin Mix V1001	10	10
Choline Bitartrate	2	2
Dyes	0.05	0.05
Energy density (kcal/g)	**3.85**	**4.73**

Table S2. Plasmid and genomic DNA initial working solution concentrations, as measured by fluorescent molecule labeling (see full description in Section 2.5 of the manuscript), used for the qPCR standard curves.

ASF taxa	Plasmid DNA (ng/µL)	Genomic DNA (ng/µL)
Clostridium sp. (ASF 356)	7.63	2.9
L. intestinalis (ASF 360)	10.2	4.84
L. murinus (ASF 361)	12.4	7.63
M. schaedleri (ASF 457)	6.06	1.97
E. plexicaudatum (ASF 492)	5.36	5.34
Pseudoflavonifractor sp. (ASF 500)	8.17	5.18
Clostridium sp. (ASF 502)	10.2	3.26
P. goldsteinii (ASF 519)	4.67	10

Table S3. Full genome length (bp) for all ASF taxa.

ASF taxa	Full genome length (bp)*
Clostridium sp. (ASF 356)	2,900,700
L. intestinalis (ASF 360)	1,868,090
L. murinus (ASF 361)	2,109,070
M. schaedleri (ASF 457)	2,319,180
E. plexicaudatum (ASF 492)	6,104,768
Pseudoflavonifractor sp. (ASF 500)	3,658,722
Clostridium sp. (ASF 502)	6,364,766
P. goldsteinii (ASF 519)	6,862,324

* **Full genome length (bp) -** full genome length for each ASF taxon that can be assessed from http://bacteria.ensembl.org/index.html.

Table S4. Standard curve parameters and limit of detection for all ASF bacteria.

Taxon ID	qPCR efficiency		Slope		Intercept		R^2		Pearson *r* coefficient		Limit of detection*	
	Mix A	Mix B	Mix A	Mix B	Mix A	Mix B	Mix A	Mix B	Mix A	Mix B	Mix A	Mix B
356	0.84	0.69	-3.774	-4.379	41.21	43.51	0.969	0.991	-0.997	-0.999	370	6,900
360	0.87	0.97	-3.693	-3.405	37.97	32.45	0.986	0.999	-0.997	-0.999	36	8
361	0.79	0.80	-3.952	-3.917	45.84	39.47	0.924	0.991	-0.994	-0.996	2,700	58
457	0.90	0.57	-3.579	-5.131	37.24	52.01	0.975	0.990	-0.997	-0.997	14	4,900
492	0.78	0.53	-4.011	-5.399	40.99	51.45	0.977	0.998	-0.999	-0.999	280	130,000
500	0.96	0.86	-3.416	-3.696	34.62	37.02	0.980	0.942	-0.996	-0.971	20	52
502	0.96	0.80	-3.421	-3.925	34.94	38.17	0.978	0.991	-0.995	-0.998	26	260
519	0.99	0.58	-3.352	-5.064	35.58	49.86	0.974	0.987	-0.997	-0.996	10	3,000

* Limit of detection expressed as total number of bacterial cells estimated from each ASF species-specific standard curve using purified plasmid DNA and adjusted for 16 rRNA gene copy number considering five copies of the 16 rRNA gene for each ASF taxon.

Supplemental Figures

Fig. S1. CLUSTAL W multiple sequence alignment of all ASF 16S rRNA gene sequences that can be assessed in the NCBI database. Regions highlighted in gray represent the primer sequences from Sarma-Rupavtarm et al., 2004, while bold nucleotides represent the newly developed primer sets herein presented. Overlapping sequences between the two primer sets appear italicized. Asterisks highlight positions with high nucleotide sequence similarity. Note that the newly developed primers are located between the hypervariable regions 1-3 of the 16S rRNA gene and uniquely positioned in regions of low nucleotide similarities across all sequences. Alignment was produced using the Bioedit software version 7.1.7 (Bioeditor Sequence Alignment Editor, Tom Hall, Ibis Biosciences, Carlsbad, CA).

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                                                5                                                  55
gi|5163477|gb|AF157056.1|ASF  519      GGCTCAGGATGAACGCTAGCGACAGGCTTAACACATGCAAGTCGAGGGGCAGCACGATGT
gi|5163471|gb|AF157050.1|ASF  360      GGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGCTGAACCAGC
gi|5163470|gb|AF157049.1|ASF  361      GGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAAACTTCTTTAT
gi|5163476|gb|AF157055.1|ASF  457      GGCTCAGAACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCAGGGAGAAAGTCTCTTC
gi|5163473|gb|AF157052.1|ASF  356      GGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGAAAATAATTAGG
gi|5163472|gb|AF157051.1|ASF  500      GGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGAGGACCCCTGA
gi|5163475|gb|AF157054.1|ASF  492      GGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGAAGCAYATCTGC
gi|5163474|gb|AF157053.1|ASF  502      GGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGAAGCACTTTTTT
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                                           65                                                 115
gi|5163477|gb|AF157056.1|ASF  519      AGCAA---------------TACATTGGTGGCGACCGGCGCACGGGTGAGTAACGCGTAT
gi|5163471|gb|AF157050.1|ASF  360      A-GATTCACTTCGGTGATGACGCTGGGAACGCGAGCGGCGGATGGGTGAGTAACACGTGG
gi|5163470|gb|AF157049.1|ASF  361      CACCGAGTGCTTGCACTCACCGATAAAGAGTTGAGTGGCGAACGGGTGAGTAACACGTGG
gi|5163476|gb|AF157055.1|ASF  457      GGGGA--------------------TGATTAAACCGGCGCACGGGTGAGTAACACGTGA
gi|5163473|gb|AF157052.1|ASF  356      AGCTTG-------------CTTTTAATTATTTTAGCGGCGGACGGGTGAGTAACGTGTGG
gi|5163472|gb|AF157051.1|ASF  500      AGGAGTTTTCGGA--CAACTGAAGGGAATCCTTAGTGGCGGACGGGTGAGTAACGCGTGA
gi|5163475|gb|AF157054.1|ASF  492      GGAATTCCTTCGGGGAGGAAGCRGTTATGACTGAGTGGCGGACGGGTGAGTAACGCGTGG
gi|5163474|gb|AF157053.1|ASF  502      AGAACTCTTCGGA--GGGAAGAGAGGGTGACTTAGCGGCGGACGGGTGAGTAACGCGTGG
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gi|5163471|gb|AF157050.1|ASF  360         GTAACCTGCCCTAAAGTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAACAAC
gi|5163470|gb|AF157049.1|ASF  361         GCAACCTGCCCAAAAGAGGGGGATAACACTTGGAAACAGGTGCTAATACCGCATAACCAT
gi|5163476|gb|AF157055.1|ASF  457         GTGACCTGCCTTTTAGACTGGAACAACTTACCGAAAGGTGAGCTAATGCCGGATGAGTTA
gi|5163473|gb|AF157052.1|ASF  356         GCAACCTGCCTTTTACTGTGGAATAATCACTGGAAACGGTGACTAATACCGCATAC***GGTT***
gi|5163472|gb|AF157051.1|ASF  500         GTAACCTGCCTTGGAGTGGGGAATAACAGCTGGAAACAGCTGCTAATACCGCATGATATG
gi|5163475|gb|AF157054.1|ASF  492         GCAACCTGCCCCATACCGGGGGACAACAGCCGGAAACGGCTGCTAATACCGCATAC**GTTT**
gi|5163474|gb|AF157053.1|ASF  502         GCAACCTGCCTTACACAGGGGGATAACAATTAGAAATGATTGCTAATACCGCATAAGACC
                                          *  **** **    *    ** * *       ****      ***** *** **
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gi|5163477|gb|AF157056.1|ASF  519         GGG**GTTCCACATGGA**------**AATATTTGTTAA**AGAATTATCGCTGATAGATGGGCATGC
gi|5163471|gb|AF157050.1|ASF  360         AATA***GCTGCATGGCTATTGC***TTAAAAGGCGGCGAAAGCTGTCGCTAAAGGATGGACCCGC
gi|5163470|gb|AF157049.1|ASF  361         AGTTACCGCATGGTAACTA**TGTAAAAGGT-GGCTATGCTA**CCGCTTTTGGATGGGCCCGC
gi|5163476|gb|AF157055.1|ASF  457         TATAA**GTGCATGTTTATATAGGAAAAGTT**GGGGAGACCTGACGCTGAAAGATGGACTCGC
gi|5163473|gb|AF157052.1|ASF  356         **CTTAGGAGGCATCTT-----CTAA**GAAAGAAAGGATTTATTCGGTAAAAGATGGGCCCGC
gi|5163472|gb|AF157051.1|ASF  500         TCTGTGTCGCATGGC-----ACT***GGAC-ATC*****AAAGATTTATCGCT**CTGAGATGGACTCGC
gi|5163475|gb|AF157054.1|ASF  492         **TTAAAACCGCATGGT-----TTTA**AA--AAGAAAACTCCGGTGGTATGGGATGGGCCCGC
gi|5163474|gb|AF157053.1|ASF  502         CCGGTACCGCATGGT-----ACAGA***G--GTAAAAACTGAGGTGGTGTAA**GATGGGCCCGC
                                                                                * *    ***** *  **
                                            245                                               295
gi|5163477|gb|AF157056.1|ASF  519         GTTCCATTAGATAGTTGGTGAGGTAACGGCTCACCAAGTCCACGATGGATAGGGGTTCTG
gi|5163471|gb|AF157050.1|ASF  360         GGTGCATTAGCTAGTTGGTAAGGTAATGGCTTACCAAGGCGACGATGCATAGCCGAGTTG
gi|5163470|gb|AF157049.1|ASF  361         GGCGCATTAGCTAGTTGGTGGGGTAAAGGCTTACCAAGGCAATGATGCGTAGCCGAACTG
gi|5163476|gb|AF157055.1|ASF  457         GTCCCATTAGCTAGTTGGTAGGGTAATGGCCTACCAAGGCGACGATGGGTAGCCGGCCTG
gi|5163473|gb|AF157052.1|ASF  356         ATCTGATTAGCTAGTTGGTGAGATAATAGCCCACCAAGGCAACGATCAGTAGCCGACCTG
gi|5163472|gb|AF157051.1|ASF  500         GTCTGATTAGCTAGTTGGCGGGGTAACGGCCCACCAAGGCGACGATCAGTAGCCGGACTG
gi|5163475|gb|AF157054.1|ASF  492         GTCTGATTAGCTGGTTGGYGGGGTAACGGCCCACCAAGGCGACGATCAGTAGCCGGCCTG
gi|5163474|gb|AF157053.1|ASF  502         GTCTGATTAGGTAGTTGGTGGGGTAGAAGCCTACCAAGCCGACGATCAGTAGCCGACCTG
                                               ***** * *****   * **   **  ****** * * ***   ***  *   **
                                            305                                               355
gi|5163477|gb|AF157056.1|ASF  519         AGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAG
gi|5163471|gb|AF157050.1|ASF  360         AGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAG
gi|5163470|gb|AF157049.1|ASF  361         AGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAG
gi|5163476|gb|AF157055.1|ASF  457         AGAGGGTGGCCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAG
gi|5163473|gb|AF157052.1|ASF  356         AGAGGGTGACCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAG
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gi|5163475|gb|AF157054.1|ASF  492         AGAGGGCGGCCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAG
gi|5163474|gb|AF157053.1|ASF  502         AGAGGGCGACCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAG
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gi|5163470|gb|AF157049.1|ASF  361      TAGGGAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGGGTGAAGAAGG
gi|5163476|gb|AF157055.1|ASF  457      TGGGGAATTTTGCGCAATGCTCGTAAGAGTGACGCAGCGACGCCGCGTGAATGACGAAGG
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gi|5163474|gb|AF157053.1|ASF  502      TGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCGACGCCGCGTGAGTGAGGAAGT
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gi|5163477|gb|AF157056.1|ASF  519      ATCTATGGTTTGTAAACTTCTTTTATATGGGAA-----TAAAGTGAGGAACGTGTTCCTT
gi|5163471|gb|AF157050.1|ASF  360      TTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCCCTA
gi|5163470|gb|AF157049.1|ASF  361      TCTTCGGATCGTAAAACCCTGTTGTTAGAGAAGAAAGTGCGTGAGAGTAACTGTTCACGT
gi|5163476|gb|AF157055.1|ASF  457      CCTTCGGGTCGTAAAGTTCTTTCGACAGGGAAGAAAATGCCTATAAGTAACTGTGTATGT
gi|5163473|gb|AF157052.1|ASF  356      TTTTCGGATTGTAAACTTCTATCAATAGG----------------------GAAGAAAGA
gi|5163472|gb|AF157051.1|ASF  500      CTTTCGGGTTGTAAACTTCTTTTCTCAGG----------------------GACGAAGCA
gi|5163475|gb|AF157054.1|ASF  492      ACCTCGGTATGTAAAGCTCTATCAG---C----------------------AGGGAAGAA
gi|5163474|gb|AF157053.1|ASF  502      ATTTCGGTATGTAAAGCTCTATCAG---C----------------------AGGGAAGAA
                                            *  *      **      *
                                                 485                                                 535
gi|5163477|gb|AF157056.1|ASF  519      TTTGTATGTACCATATGAATAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACG
gi|5163471|gb|AF157050.1|ASF  360      TTTGACGGTAATCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
gi|5163470|gb|AF157049.1|ASF  361      TTCGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
gi|5163476|gb|AF157055.1|ASF  457      ATTGACGGTACCTGTATAAGCAGCCCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACG
gi|5163473|gb|AF157052.1|ASF  356      AATGACGGTACCTAAATAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
gi|5163472|gb|AF157051.1|ASF  500      AGTGACGGTACCTGAGGAATAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
gi|5163475|gb|AF157054.1|ASF  492      AGTGACAGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
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gi|5163470|gb|AF157049.1|ASF  361      TAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGGGAACGCAGGCGGTCTTTTAAG
gi|5163476|gb|AF157055.1|ASF  457      GAGGGGGCGAGCGTTGTTCGGAGTGACTGGGCGTAAAGAGCACGTAGGCGGTGTTGTAAG
gi|5163473|gb|AF157052.1|ASF  356      TAGGGGGCAAGCGTTATCCGGAATTACTGGGTGTAAAGGGAGAGTAGGCGGCAAGGTAAG
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gi|5163473|gb|AF157052.1|ASF  356      CGATATGTGAAAGCCTTAAGCTTAACTTAAGGATAGCATAACGAACTATCTAGCTAGAGT
gi|5163472|gb|AF157051.1|ASF  500      TCAGATGTGAAAACCACGGGCTCAACCTGTGGCCTGCATTTGAAACTGTAGTTCTTGAGT
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gi|5163470|gb|AF157049.1|ASF  361      GCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAAC
gi|5163476|gb|AF157055.1|ASF  457      ATCAGAGAGGATTGCAGAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGGAGGAAT
gi|5163473|gb|AF157052.1|ASF  356      ACAGGAGAGGAAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAAGAAC
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gi|5163475|gb|AF157054.1|ASF  492      GTCGGAGGGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAAC
gi|5163474|gb|AF157053.1|ASF  502      GTCGGAGAGGCAAGTGGAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGGAGGAAC
                                              * **      ***  *   *********** ***** *******   *  ***
                                                 725                                                 775
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gi|5163475|gb|AF157054.1|ASF  492      ACCAGTGGCGAAGGCGGCTTACTGGACGATCACTGACGCTGAGGCTCGAAAGCGTGGGGA
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                                                 785                                                 835
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gi|5163470|gb|AF157049.1|ASF  361      GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAAGTGTTGGA
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gi|5163475|gb|AF157054.1|ASF  492      GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATACTAGGTGTCGGC
gi|5163474|gb|AF157053.1|ASF  502      GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGACTACTAGGTGTCGGG
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gi|5163470|gb|AF157049.1|ASF  361      GGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCAATAAGCATTCCGCCTGGGGAGTACGAC
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                                                 905                                                                 955
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gi|5163472|gb|AF157051.1|ASF  500      CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTT
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gi|5163470|gb|AF157049.1|ASF  361      TAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTTTGACAATCCTAGAGA
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gi|5163473|gb|AF157052.1|ASF  356      TAATTCGAAGCAACGCGAAGAACCTTACCAAGGCTTGACATCCTCTTGACGAATGTAGAG
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gi|5163470|gb|AF157049.1|ASF  361      TAGGAC------TTTCCCTTCGGGGACAAAATGACAGGTGGTGCATGGTTGTCGTCAGCT
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gi|5163474|gb|AF157053.1|ASF  502      CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTACCTTCAGTAGCCA
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gi|5163470|gb|AF157049.1|ASF  361      GCATTA--AGTTGGGCACTCTAGCAAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
gi|5163476|gb|AF157055.1|ASF  457      ACGGTTGAAGCTGAGCACTCTGGAGAGACTGCCAGCGATAAGCTGGAGGAAGGTGGGGAC
gi|5163473|gb|AF157052.1|ASF  356      GCAGGTAGAGCTGGGCACTCTGGAGAGACTGCCGTGGATAACACGGAGGAAGGTGGGGAT
gi|5163472|gb|AF157051.1|ASF  500      C-------GCAAGAGCACTCTAGCGAGACTGCCGTTGACAAAACGGAGGAAGGTGGGGAC
gi|5163475|gb|AF157054.1|ASF  492      GCGGATAGAGCCGGGCACTCTGGGGAGACTGCCGGGGACAACCCGGAGGAAGGCGGGGAC
gi|5163474|gb|AF157053.1|ASF  502      GCATTTAG-GATGGGCACTCTGGAGGGACTGCCAGGGACAACYTGGAGGAAGGTGGGGAT
                                                  * * *****     *******   *  *     ******** *****
                                                1205                                                    1255
gi|5163477|gb|AF157056.1|ASF  519      GACGTCAAATCAGCACGGCCCTTACATCCGGGGCGACACACGTGTTACAATGGTGGGGAC
gi|5163471|gb|AF157050.1|ASF  360      GACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGTAC
gi|5163470|gb|AF157049.1|ASF  361      GACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTAC
gi|5163476|gb|AF157055.1|ASF  457      GACGTCAAGTCATCATGGCCCTTATGTCCAGGGCTACACACGTGCTACAATGGCATAATC
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gi|5163471|gb|AF157050.1|ASF  360      AACGAGAAGCGAGC-CTGCGAAGGCAAGCGGATCTCTTAAAGCTGTTCTCAGTTCGGACT
gi|5163470|gb|AF157049.1|ASF  361      AACGAGTCGCAAGA-CCGCGAGGTTTAGCAAATCTCTTAAAGCCGTTCTCAGTTCGGATT
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gi|5163475|gb|AF157054.1|ASF  492      AGAGGGAAGCGAAG-CCGCGAGGTGGAGCAAACCCCAGAAATGGCGTCTCAGTTCGGACT
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gi|5163470|gb|AF157049.1|ASF  361      GTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCA-TGCC
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gi|5163475|gb|AF157054.1|ASF  492      GCAGCCTGCAACTCGGCTGCACGAAGCCGGAATCGCTAGTAATCGCAGATCAGCA-TGCT
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                                        * ** ******* **  * *  ****  *** **************  *****    *
                                                1385                                                1435
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gi|5163471|gb|AF157050.1|ASF  360      GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGAAGTCTGCAAT
gi|5163470|gb|AF157049.1|ASF  361      GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC
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gi|5163474|gb|AF157053.1|ASF  502      GCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGTAAC
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gi|5163470|gb|AF157049.1|ASF  361      ACCCAAAGCCGGTGGGGTAACCTTT----TGGAGCCAGCCGTCTAAGGTGGGACAGATGA
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gi|5163475|gb|AF157054.1|ASF  492      GCCCGAAGTCAGTGGCCCAACCGCAA----GGAGGGAGCTGCCGAAGGCAGGTCCGGTGA
gi|5163474|gb|AF157053.1|ASF  502      GCCCGAAGCCGGTGACCCAACCTAASC--AGGAGGGAGCCGTCGAAGGCGGGACGGATGA
                                         **  *** *  *                              * * * **         * *
                                                1505                          1525
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gi|5163476|gb|AF157055.1|ASF  457      CTGGGGTGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCG
gi|5163473|gb|AF157052.1|ASF  356      CTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCG
gi|5163472|gb|AF157051.1|ASF  500      TTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCG
gi|5163475|gb|AF157054.1|ASF  492      CTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCG
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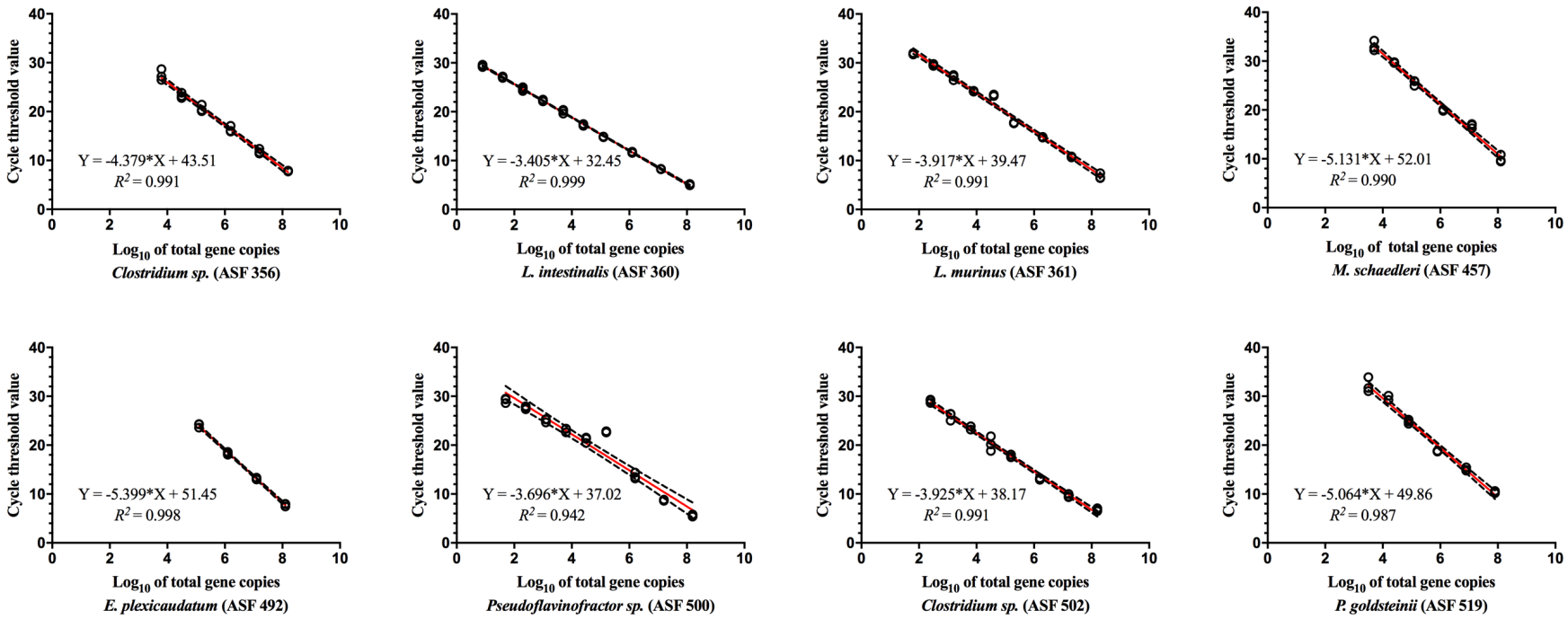


Fig. S2. Linear regression model for SYBR Green Master Mix B depicting the standard curve parameters for ASF quantification. Standard curves were prepared using serial dilutions of purified vector-free plasmid DNA containing the specific16S rRNA gene sequence for each ASF taxon and used to determine the limit of detection and efficiency of each reaction. The predicted linear model line (red) and estimated equation are shown for each ASF taxon based on the $\log_{10}$ of total 16S rRNA gene copies, along with dotted lines representing the 95% confidence interval bands. Triplicates of each plasmid concentration were used to determine precision in quantification (open black circles). R-squared values are also shown for each curve. The final number for the $\log_{10}$ of total 16S rRNA gene copies for each ASF bacterium was calculated considering five copies of this gene per bacterial genome.

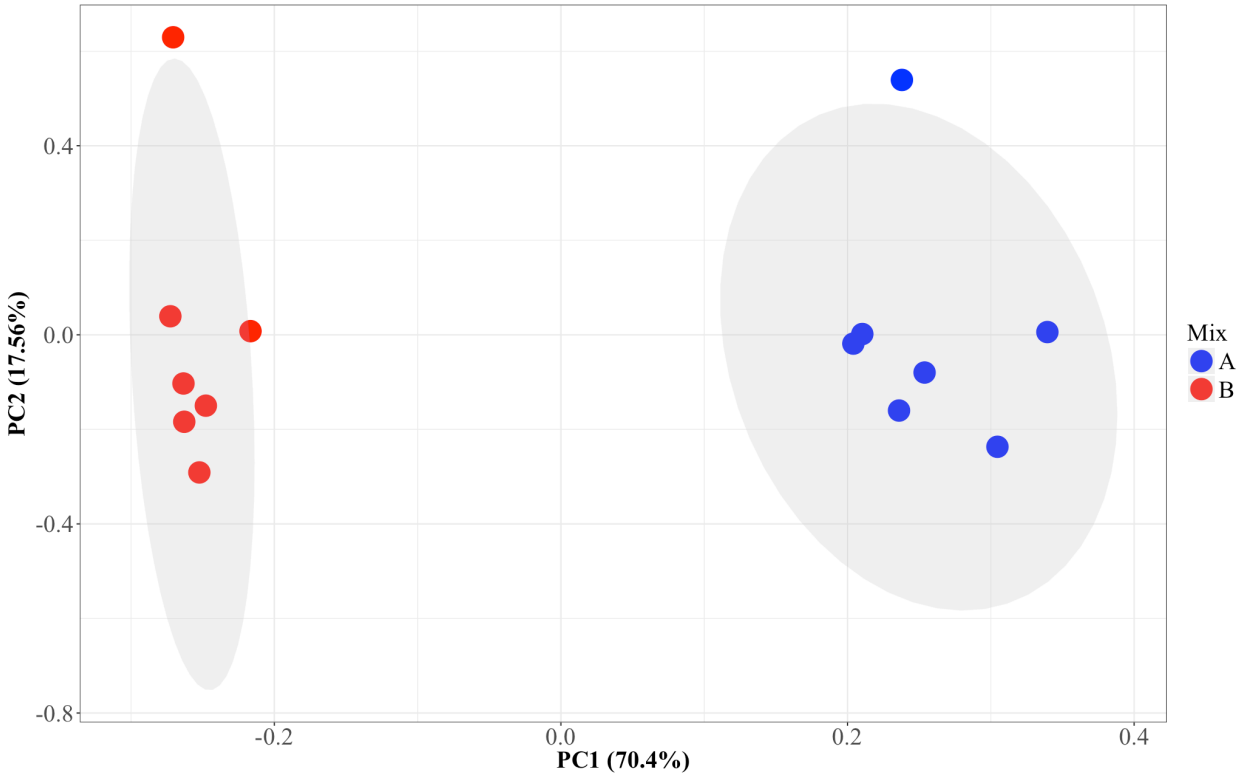


Fig. S3. PCA plot depicting how the ASF community clusters when comparing the $\log_{10}$ of total 16S rRNA gene copies per gram of cecal contents between mouse genotypes (i.e., Mix A in blue and Mix B in red). The x- and y-axes indicate the principal components 1 (PC1) and 2 (PC2) and include the percent of variance explained by each PC. Each dot in the PCA plot represents an individual animal and its respective ASF member abundances. Gray shaded ellipses represent dispersion of the data points within treatments and were calculated based on a multivariate T distribution. All qPCRs were run in duplicate for both master mixes. Estimated ASF abundances were achieved using the corresponding plasmid standard curve for each mix.

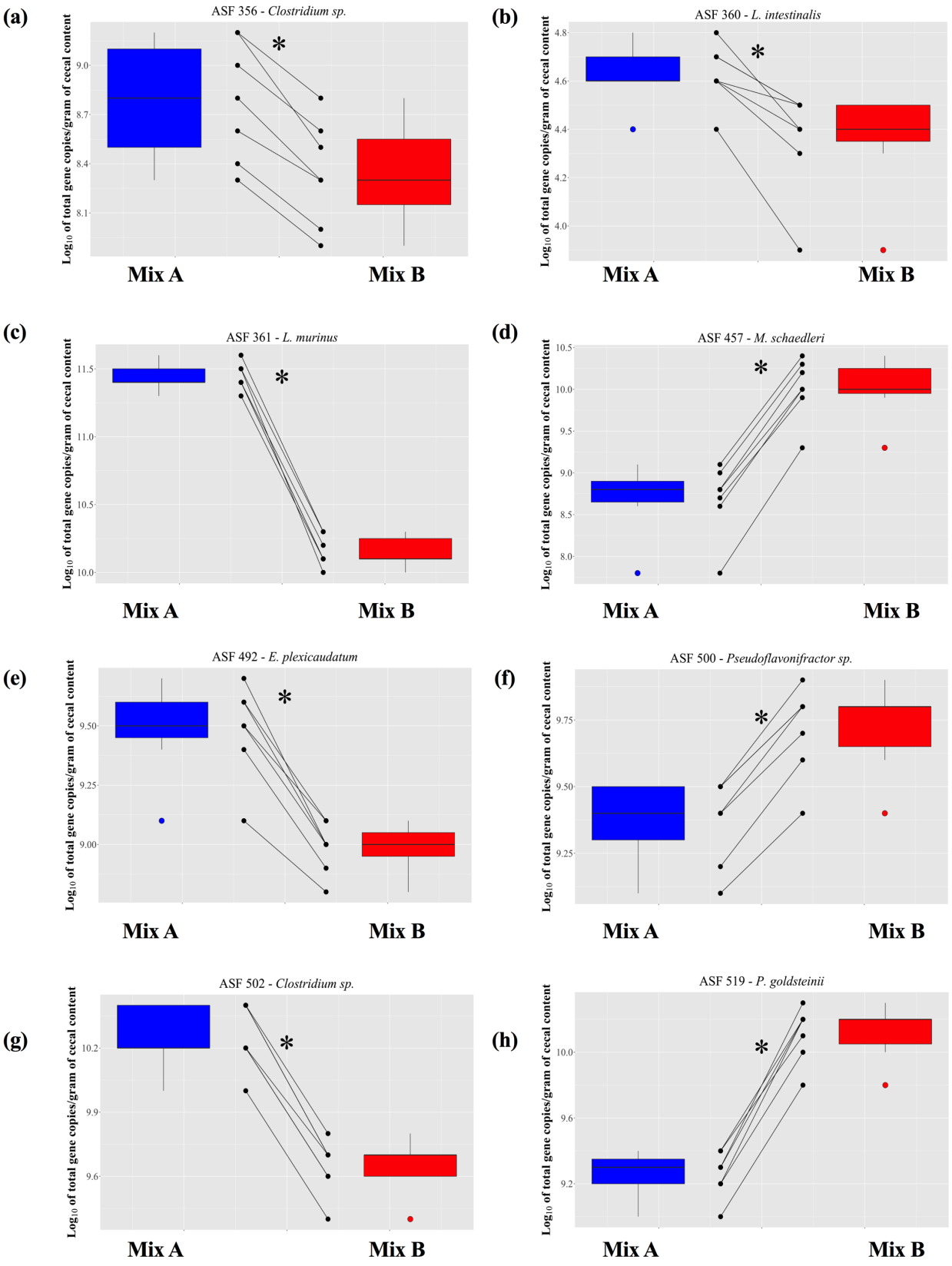

(a)
ASF 356 - Clostridium sp.
(b)
ASF 360 - L. intestinalis
(c)
ASF 361 - L. murinus
(d)
ASF 457 - M. schaedleri
(e)
ASF 492 - E. plexicaudatum
(f)
ASF 500 - Pseudoflavonifractor sp.
(g)
ASF 502 - Clostridium sp.
(h)
ASF 519 - P. goldsteinii
Log10 of total gene copies/gram of cecal content
Mix A
Mix B
*

Fig. S4. Box-and-whisker plots showing quantification of the ASF bacterial species, based on the 16S rRNA copy number, in cecal contents of gnotobiotic C57BL/6 (n = 7) mice when comparing two qPCR SYBR Green master mixes (Mix A in blue and Mix B in red; Panels a-h). In each graph, the y-axis indicates the ASF taxon $\log_{10}$ of total 16S rRNA gene copies per gram of cecal contents, and the x-axis depicts each ASF member taxonomy and identification number. Whiskers depict the 1.5 x IQR (i.e., IQR = interquartile range as the distance between the first and third quartiles), the horizontal bar in the middle of the box represents the median value, and red and blue circles above or below whiskers indicate possible outliers. Black circles (in the middle of each graph) indicate each individual observation, and the black lines show the directionality of change in bacterial abundance across all samples for each ASF member when results from the two Master Mixes were compared. Asterisks refer to the degree of significance for the difference in bacterial abundance as determined by the non-parametric Wilcoxon matched-pairs signed rank test using a two-tailed distribution for p-value calculations (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$). All qPCRs were run in duplicate for both SYBR Green mixes.

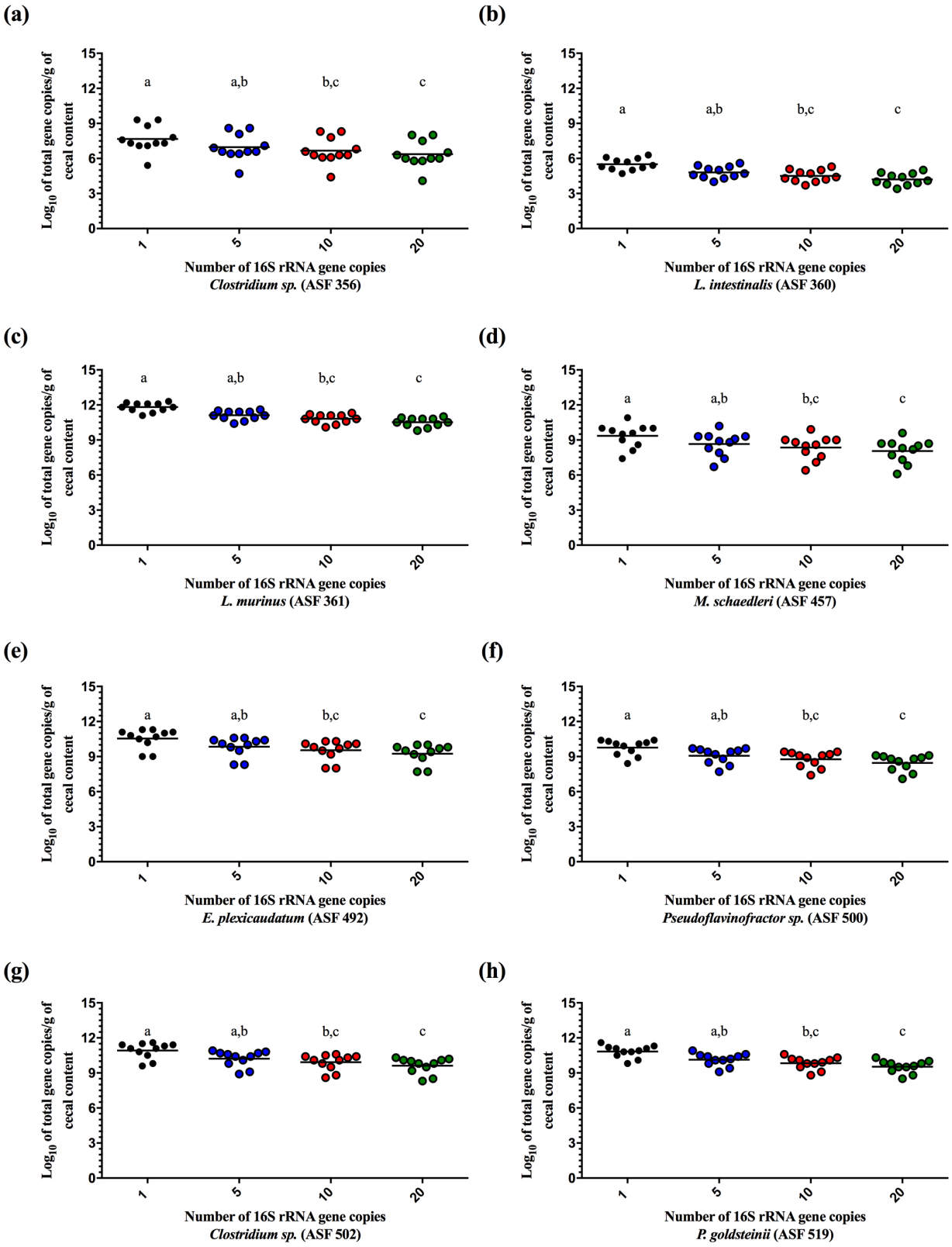

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Log10 of total gene copies/g of cecal content
a
a,b
b,c
c
1
5
10
20
Number of 16S rRNA gene copies
Clostridium sp. (ASF 356)
L. intestinalis (ASF 360)
L. murinus (ASF 361)
M. schaedleri (ASF 457)
E. plexicaudatum (ASF 492)
Pseudoflavinofractor sp. (ASF 500)
Clostridium sp. (ASF 502)
P. goldsteinii (ASF 519)

Fig. S5. Dot plots showing quantification of each ASF bacterial species based on either 1, 5, 10 or 20 copies of the 16S rRNA gene in cecal contents of gnotobiotic C3H/HeN mice (n = 11). The y-axis indicates the ASF taxon $\log_{10}$ of total 16S rRNA gene copies per gram of cecal contents. The x-axis depicts the number of 16S rRNA gene copies used to estimate the total bacterial abundance (y-axis values) by ASF member using the linear equation model generated with the plasmid standard curves as shown in Table S1 (Panels a-h). Asterisks refer to the degree of significance for the difference between the estimated bacterial abundances as determined by the Friedman's test (non-parametric Anova using matched row values across groups) followed by a pairwise comparison across all groups using the Dunn's test. Differing letters depicted in the figures indicate significant differences across groups ($p < 0.05$). All qPCRs were run in duplicate using SYBR Green Master Mix A. Estimated ASF abundances were achieved using the corresponding plasmid standard curve. Each dot in all plots represents an individual mouse.

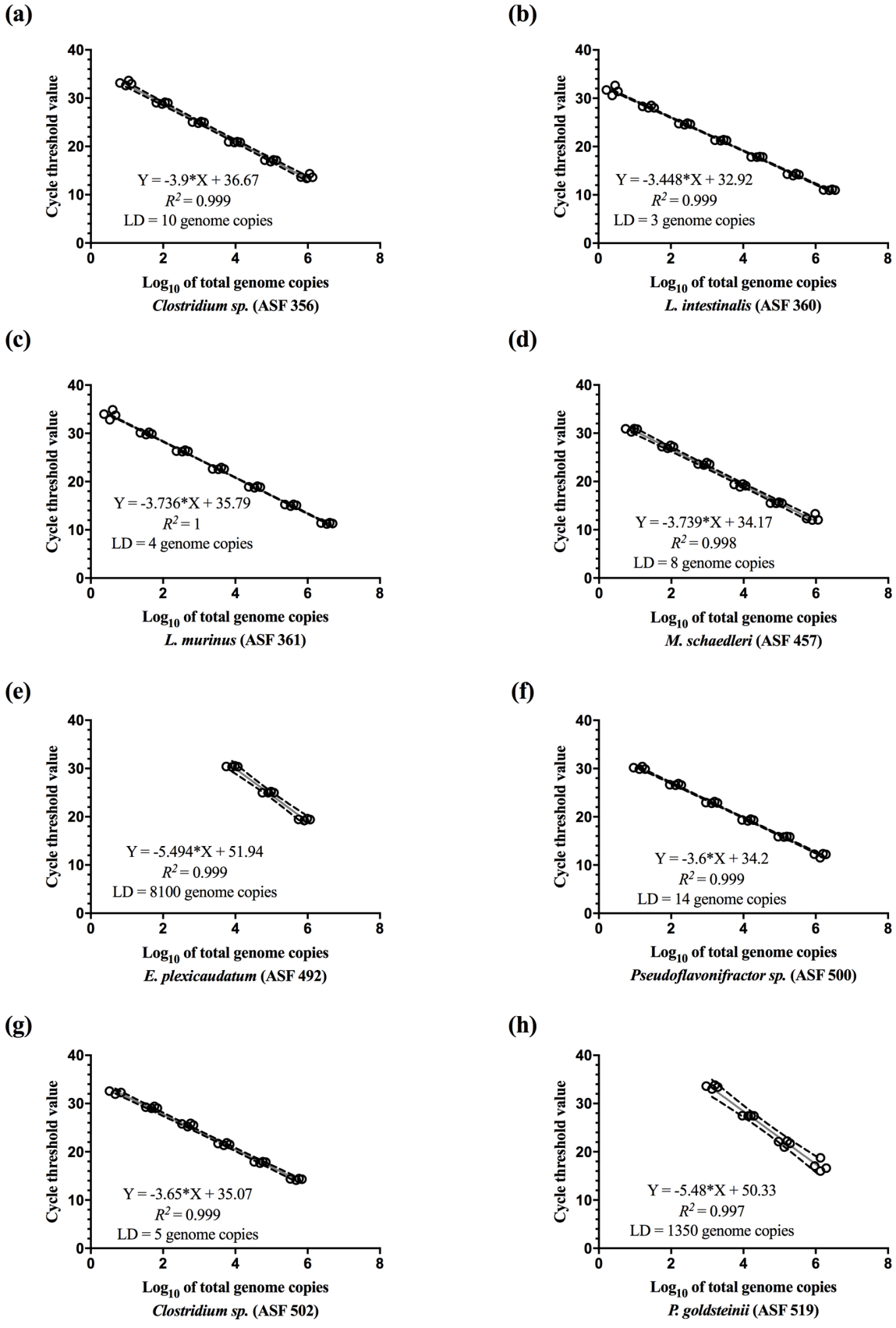

(a)
Cycle threshold value
Y = -3.9*X + 36.67
R^2 = 0.999
LD = 10 genome copies
Log10 of total genome copies
Clostridium sp. (ASF 356)
(b)
Cycle threshold value
Y = -3.448*X + 32.92
R^2 = 0.999
LD = 3 genome copies
Log10 of total genome copies
L. intestinalis (ASF 360)
(c)
Cycle threshold value
Y = -3.736*X + 35.79
R^2 = 1
LD = 4 genome copies
Log10 of total genome copies
L. murinus (ASF 361)
(d)
Cycle threshold value
Y = -3.739*X + 34.17
R^2 = 0.998
LD = 8 genome copies
Log10 of total genome copies
M. schaedleri (ASF 457)
(e)
Cycle threshold value
Y = -5.494*X + 51.94
R^2 = 0.999
LD = 8100 genome copies
Log10 of total genome copies
E. plexicaudatum (ASF 492)
(f)
Cycle threshold value
Y = -3.6*X + 34.2
R^2 = 0.999
LD = 14 genome copies
Log10 of total genome copies
Pseudoflavonifractor sp. (ASF 500)
(g)
Cycle threshold value
Y = -3.65*X + 35.07
R^2 = 0.999
LD = 5 genome copies
Log10 of total genome copies
Clostridium sp. (ASF 502)
(h)
Cycle threshold value
Y = -5.48*X + 50.33
R^2 = 0.997
LD = 1350 genome copies
Log10 of total genome copies
P. goldsteinii (ASF 519)

Fig. S6. Linear regression model depicting the standard curve parameters for ASF quantification based on total genome copies of each taxon using molecular grade water as a reaction matrix. Standard curves were prepared using ten-fold serial dilutions of purified genomic DNA in molecular grade water from each ASF bacterium. The predicted linear model line (gray), estimated equation, R-squared values and limit of detection (LD) are shown for each ASF bacterium based on the $\log_{10}$ of total genome copies calculated using the total genome mass for each taxon and the total number of base pairs per bacterial genome. Also shown are the 95% confidence interval bands (black dotted lines) and serial dilution points (open black circles). The final number for the $\log_{10}$ of total genome copies for each ASF bacterium was calculated without correcting for the number of 16S rRNA copies. All reactions were run in quadruplicate using SYBR Green Master Mix B.

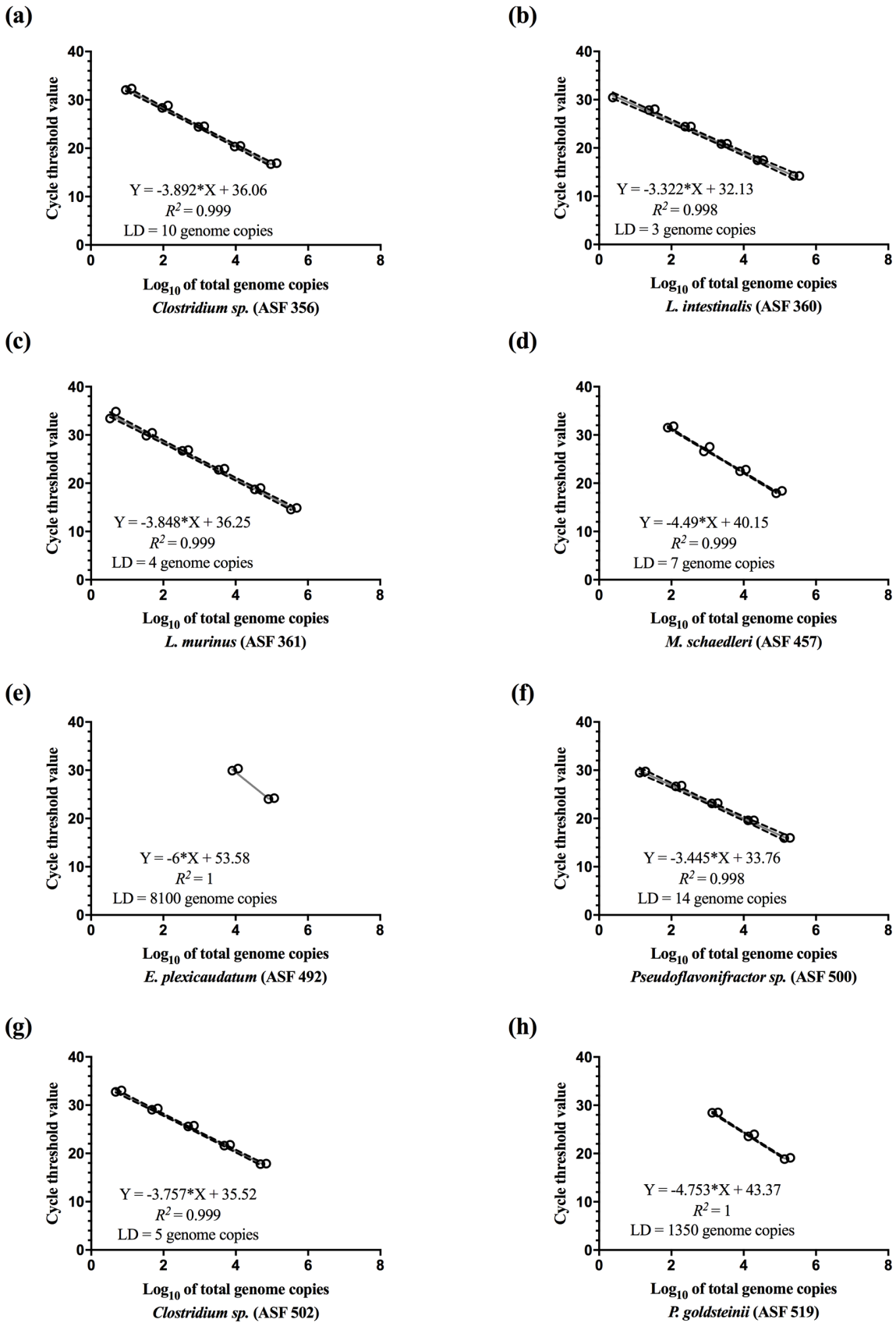

(a)
Cycle threshold value
Y = -3.892*X + 36.06
R^2 = 0.999
LD = 10 genome copies
Log10 of total genome copies
Clostridium sp. (ASF 356)
(b)
Cycle threshold value
Y = -3.322*X + 32.13
R^2 = 0.998
LD = 3 genome copies
Log10 of total genome copies
L. intestinalis (ASF 360)
(c)
Cycle threshold value
Y = -3.848*X + 36.25
R^2 = 0.999
LD = 4 genome copies
Log10 of total genome copies
L. murinus (ASF 361)
(d)
Cycle threshold value
Y = -4.49*X + 40.15
R^2 = 0.999
LD = 7 genome copies
Log10 of total genome copies
M. schaedleri (ASF 457)
(e)
Cycle threshold value
Y = -6*X + 53.58
R^2 = 1
LD = 8100 genome copies
Log10 of total genome copies
E. plexicaudatum (ASF 492)
(f)
Cycle threshold value
Y = -3.445*X + 33.76
R^2 = 0.998
LD = 14 genome copies
Log10 of total genome copies
Pseudoflavonifractor sp. (ASF 500)
(g)
Cycle threshold value
Y = -3.757*X + 35.52
R^2 = 0.999
LD = 5 genome copies
Log10 of total genome copies
Clostridium sp. (ASF 502)
(h)
Cycle threshold value
Y = -4.753*X + 43.37
R^2 = 1
LD = 1350 genome copies
Log10 of total genome copies
P. goldsteinii (ASF 519)

Fig. S7. Linear regression model depicting the standard curve parameters for ASF quantification based on total genome copies of each taxon using DNA extracted from feces of germ-free mice as a reaction matrix. Standard curves were prepared using ten-fold serial dilutions of purified genomic DNA from each ASF in DNA extracted from the feces of germ-free C3H/HeN and C57BL/6 mice (mixed 1:1 for each genotype) to verify that the reaction matrix (i.e., DNA extracted from feces versus molecular grade water; Fig. S7) did not affect assay performance. The predicted linear model line (gray), estimated equation, R-squared values and limit of detection (LD) are shown for each ASF bacterium based on the $\log_{10}$ of total genome copies calculated using the total genome mass for each taxon and the total number of base pairs. Also shown are the 95% confidence interval bands (black dotted lines) and serial dilution points (open black circles). The final number for the $\log_{10}$ of total genome copies for each ASF bacterium was calculated without correcting for the number of 16S rRNA copies. All reactions were run in duplicate using the SYBR Green Master Mix B.

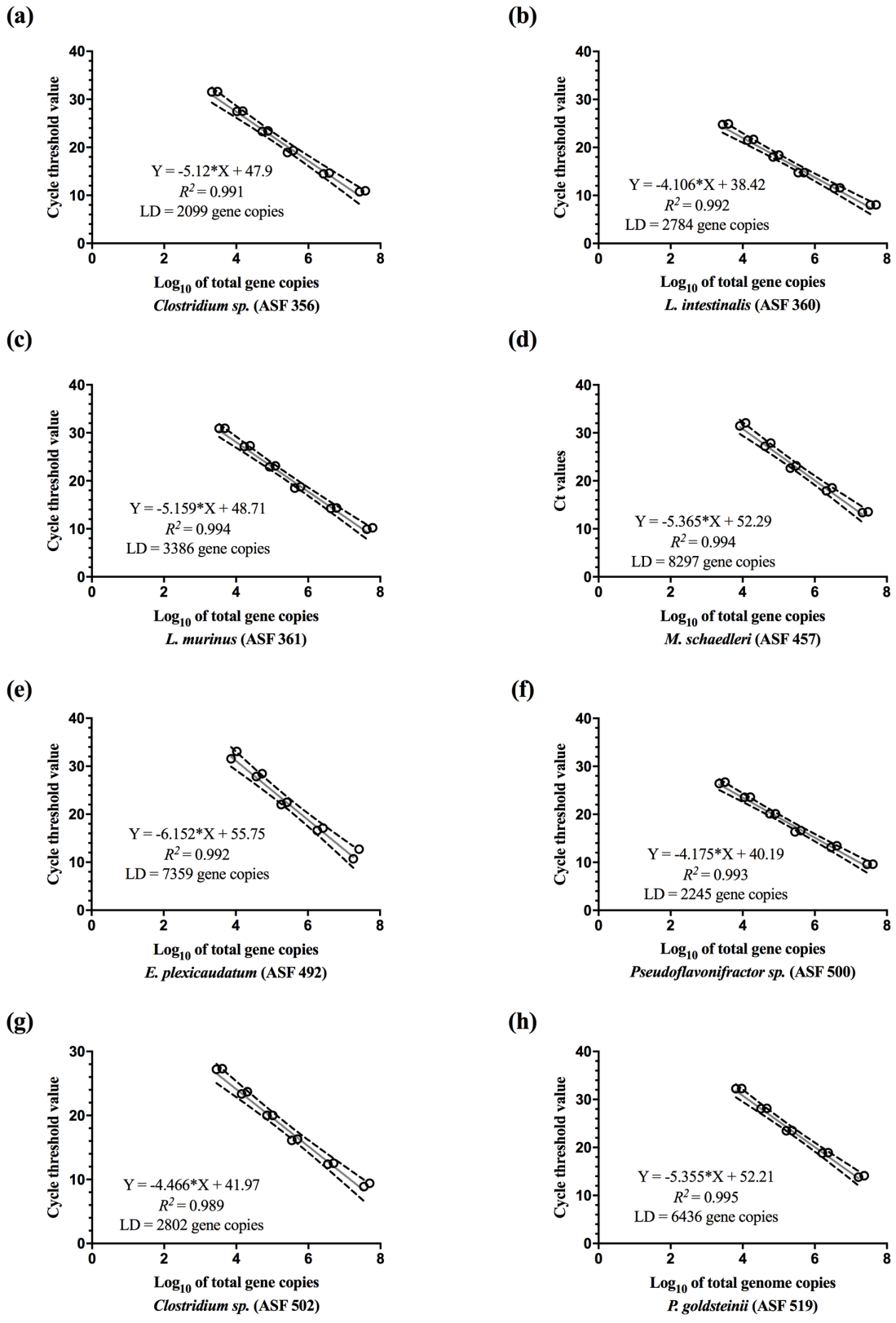

(a)
Cycle threshold value
Y = -5.12*X + 47.9
R^2 = 0.991
LD = 2099 gene copies
Log10 of total gene copies
Clostridium sp. (ASF 356)
(b)
Cycle threshold value
Y = -4.106*X + 38.42
R^2 = 0.992
LD = 2784 gene copies
Log10 of total gene copies
L. intestinalis (ASF 360)
(c)
Cycle threshold value
Y = -5.159*X + 48.71
R^2 = 0.994
LD = 3386 gene copies
Log10 of total gene copies
L. murinus (ASF 361)
(d)
Ct values
Y = -5.365*X + 52.29
R^2 = 0.994
LD = 8297 gene copies
Log10 of total gene copies
M. schaedleri (ASF 457)
(e)
Cycle threshold value
Y = -6.152*X + 55.75
R^2 = 0.992
LD = 7359 gene copies
Log10 of total gene copies
E. plexicaudatum (ASF 492)
(f)
Cycle threshold value
Y = -4.175*X + 40.19
R^2 = 0.993
LD = 2245 gene copies
Log10 of total gene copies
Pseudoflavonifractor sp. (ASF 500)
(g)
Cycle threshold value
Y = -4.466*X + 41.97
R^2 = 0.989
LD = 2802 gene copies
Log10 of total gene copies
Clostridium sp. (ASF 502)
(h)
Cycle threshold value
Y = -5.355*X + 52.21
R^2 = 0.995
LD = 6436 gene copies
Log10 of total genome copies
P. goldsteinii (ASF 519)

Fig. S8. Linear regression model depicting the standard curve parameters for ASF quantification based on plasmid DNA standard curves and the 16S rRNA copy number per ASF taxon using DNA extracted from feces of germ-free mice as a reaction matrix. Standard curves were prepared using ten-fold serial dilutions of purified vector-free plasmid DNA containing the 16S rRNA gene sequence of each ASF bacterium in DNA extracted from the feces of germ-free C3H/HeN and C57BL/6 mice (mixed 1:1 for each genotype) to verify that the reaction matrix (i.e., DNA extracted from feces versus molecular grade water; Fig. S2) did not affect assay performance. The predicted linear model line (gray), estimated equation, R-squared values and limit of detection (LD) are shown for each ASF bacterium based on the $\log_{10}$ of total of 16S rRNA gene copies. For the calculations, five was used as the final number of 16S rRNA gene copies per ASF bacterial genome. Also shown are the 95% confidence interval bands (black dotted lines) and serial dilution points (open black circles). All reactions were run in duplicate using SYBR Green Master Mix B. Of note, only six serial dilutions were made for each ASF plasmid solution, since the goal of this experiment was to show that the fecal germ-free matrix did not interfere with the overall detection and linearity of the reactions. Therefore, the limit of detection shown here is based on the last dilution point used and not the true limit of detection shown in Fig. S2.

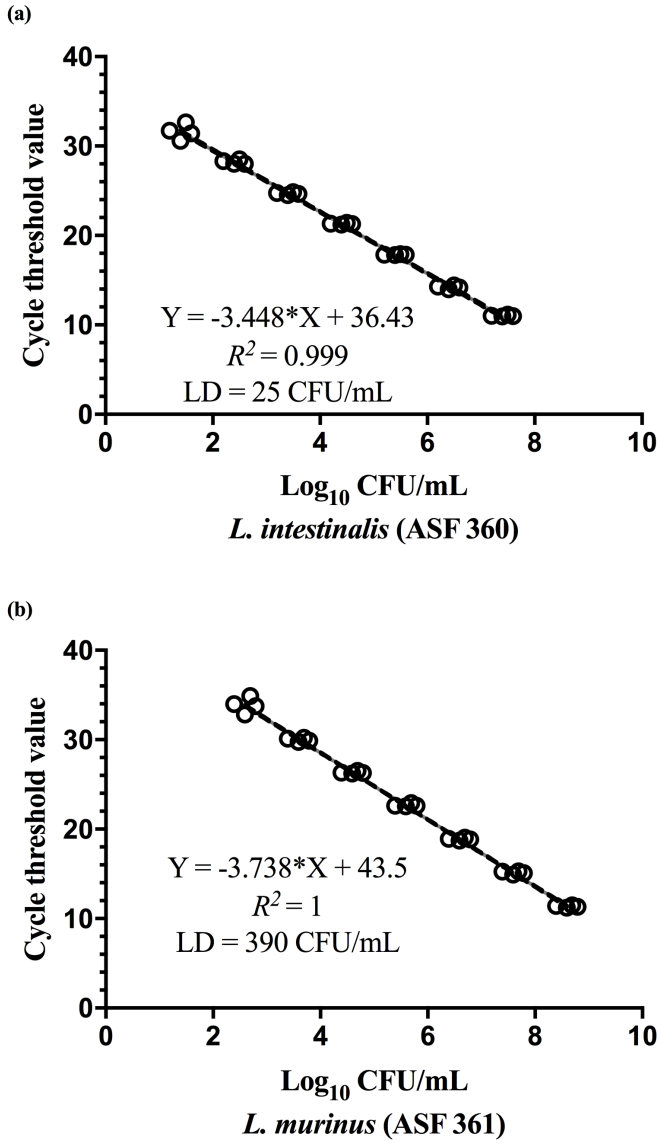


Fig. S9. Linear regression model depicting the standard curve parameters for ASF 360 and ASF 361 quantification based on $\log_{10}$ CFU/mL. Standard curves were prepared using ten-fold serial dilutions of a culture grown in tryptic soy (TS) broth. Purified genomic DNA was then extracted from one mL of each dilution for both ASF 360 and 361. The initial inoculum concentration was determined using a standard serial dilution procedure by plating ten-fold serially diluted samples in triplicate on TS agar plates (ASF 360 = 2.5×10^7 CFU/mL, ASF 361 = 3.9×10^8 CFU/mL). All growth was performed under aerobic conditions at 37°C with no shaking. The graph above depicts the predicted linear model line (gray), estimated equation, R-squared values and limit of detection for each bacterium. Also shown are the 95% confidence interval bands (black dotted lines) and serial dilution points (open black circles). All reactions were run in quadruplicate (i.e., four independent extractions per dilution) using SYBR Green Master Mix B.

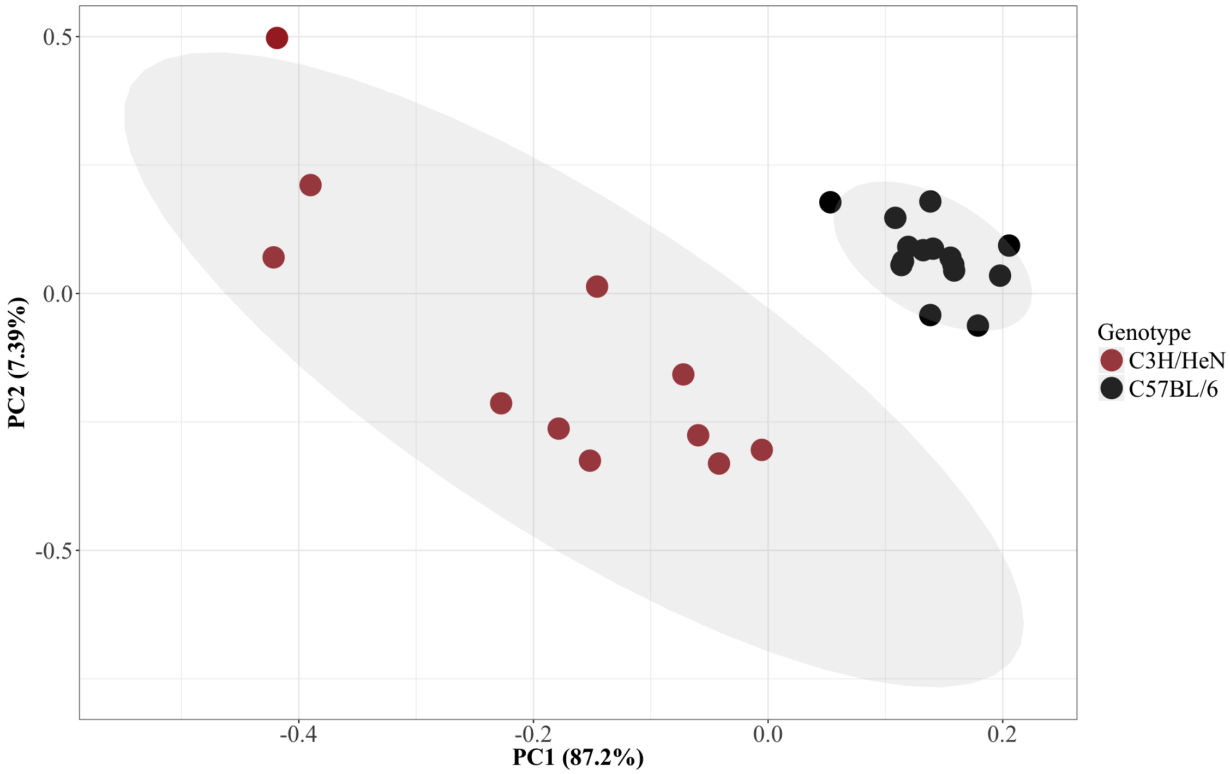
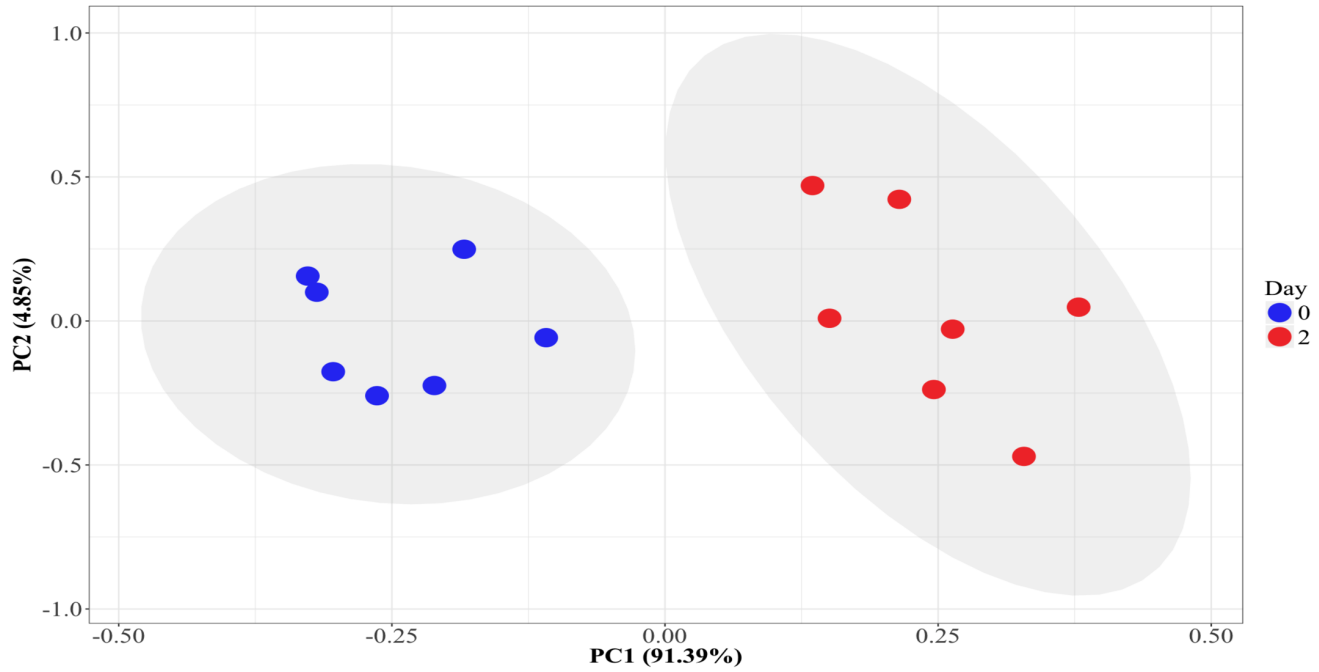


Fig. S10. PCA plot depicting how the ASF community clusters when comparing the $\log_{10}$ of total 16S rRNA gene copies per gram of cecal contents between mouse genotypes (i.e., C3H/HeN in brown and C57BL/6 in black). The x- and y-axes indicate the principal components 1 (PC1) and 2 (PC2) and include the percent of variance explained by each PC. Each dot in the PCA plot represents an individual animal and its respective ASF member abundances. Gray shaded ellipses represent dispersion of the data points within treatments and were calculated based on a multivariate T distribution. All qPCRs were run in duplicate using SYBR Green Master Mix A. Estimated ASF abundances were achieved using the corresponding plasmid standard curve.

(a)


1.0
0.5
0.0
-0.5
-1.0
PC2 (4.85%)
-0.50
-0.25
0.00
0.25
0.50
PC1 (91.39%)
Day
0
2

(b)

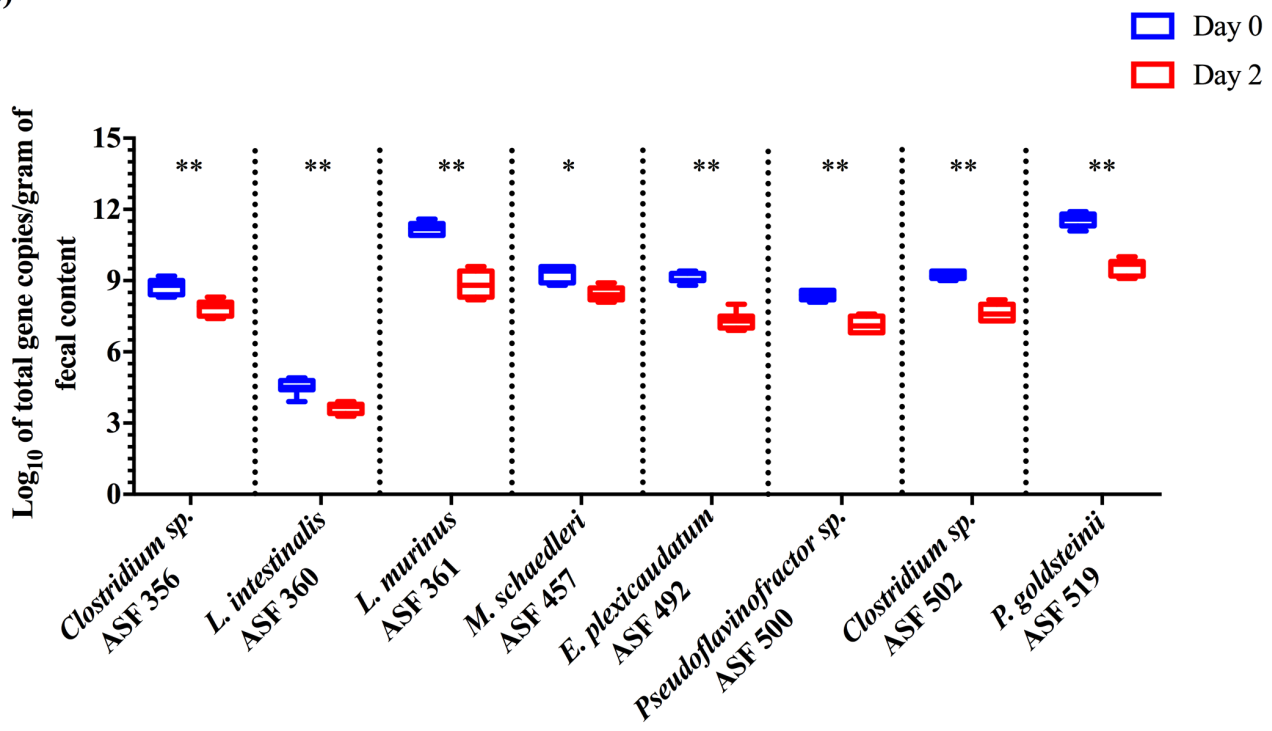

Day 0
Day 2
Log10 of total gene copies/gram of fecal content
15
12
9
6
3
0
**
**
**
*
**
**
**
**
Clostridium sp. ASF 356
L. intestinalis ASF 360
L. murinus ASF 361
M. schaedleri ASF 457
E. plexicaudatum ASF 492
Pseudoflavinofractor sp. ASF 500
Clostridium sp. ASF 502
P. goldsteinii ASF 519

Fig. S11. PCA plot depicting how the ASF community clusters when comparing the $\log_{10}$ of total 16S rRNA gene copies per gram of cecal contents between day 0 and 2 for neomycin treated mice. The x- and y-axes indicate the principal components 1 (PC1) and 2 (PC2), including the percent of variance explained by each PC. Each dot in the PCA plots represents an individual animal with its respective ASF community composition. The gray shaded areas in the plot show the dispersion of the data points within groups and were calculated based on a multivariate T distribution (Panel a). Box-and-whisker plots showing a significant decrease in the individual ASF abundances estimated using the 16S rRNA copy numbers of all bacterial species in the feces of 8 week-old gnotobiotic C57BL/6 mice (n = 7 per group) following treatment with neomycin for two consecutive days (10 mg/mL of drinking water for both antibiotics) (Panel b). The y-axis indicates the ASF taxon $\log_{10}$ of total 16S rRNA gene copies per gram of fecal content, and the x-axis depicts each ASF member taxonomy and identification number. Whiskers depict the entire range of values (min to max); the horizontal bar in the middle of the box represents the median value. Asterisks refer to the degree of significance for the difference in bacterial 16S rRNA gene copies as determined by the non-parametric Wilcoxon matched-pairs signed rank test using a one-tailed distribution for p-value calculations (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$). All qPCRs were run in duplicate using SYBR Green Master Mix A. Estimated ASF abundances were achieved using the corresponding plasmid standard curve.