

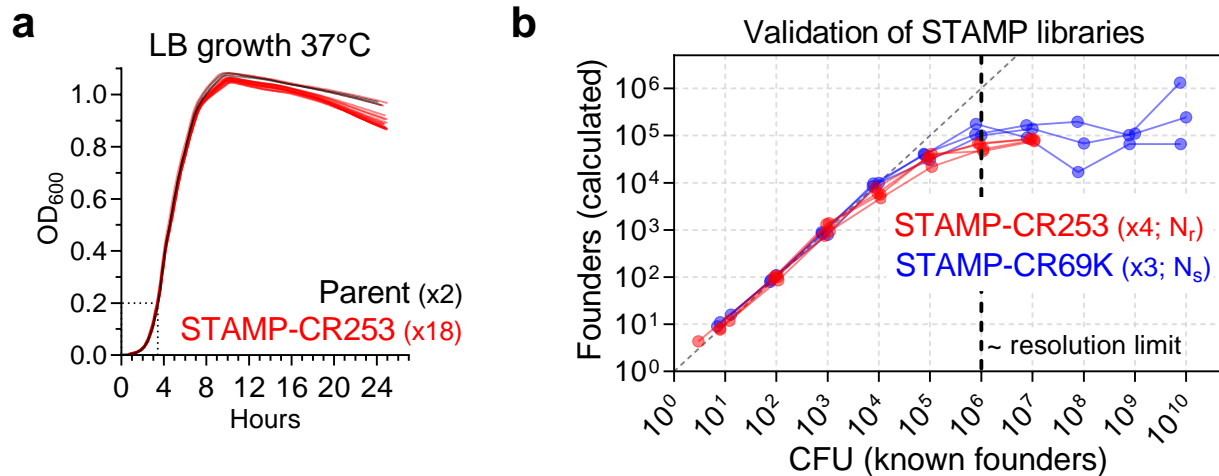
Supplementary Information

Quantitative dose-response analysis untangles host bottlenecks to enteric infection

Ian W. Campbell, Karthik Hullahalli, Jerrold R. Turner, and Matthew K. Waldor*

*mwaldor@research.bwh.harvard.edu

Supplement 1



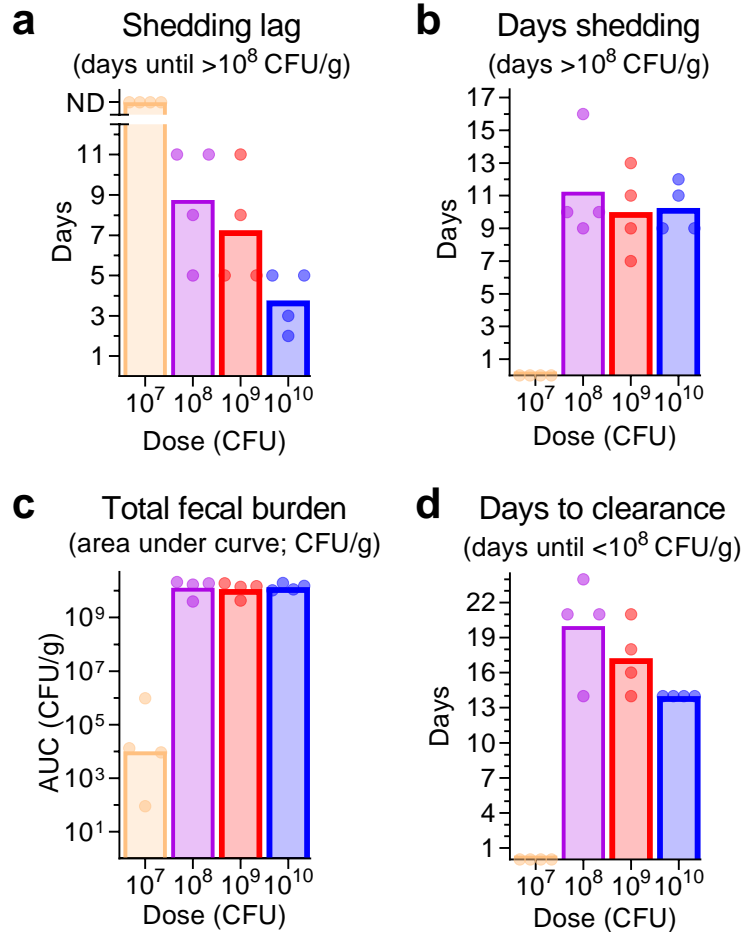
Supplemental figure 1. Validation of *Citrobacter rodentium* STAMP libraries.

(a) Growth of STAMP-CR253 library compared to the parental, untagged strain. Growth curve performed in 96 well plate reader at 37°C, shaking, in LB. 18 clones (colonies) from STAMP library and 2 from parent. Lines are means of triplicate samples.

(b) The ability to determine the size of the founding population from STAMP libraries was validated in culture by comparing the number of plated colonies (CFU; known number of founders) to the size of the founding population following processing of those plated populations (calculated founders). Without calibration, STAMP accurately determined the number of founders up to $\sim 10^4$ CFU and plateaued at $\sim 10^6$ CFU. Therefore, these values can be used to calibrate future experiments to determine the size of founding populations up to 10^6 , with increased uncertainty above 10^4 . STAMP-CR253 (253 unique barcodes) founders (N_r) measured for 4 cultures across 7 doses. STAMP-CR69K ($\sim 69,000$ unique barcodes) founders (N_s) measured for 3 cultures across 10 doses.

Source data are provided as a Source Data file.

Supplement 2

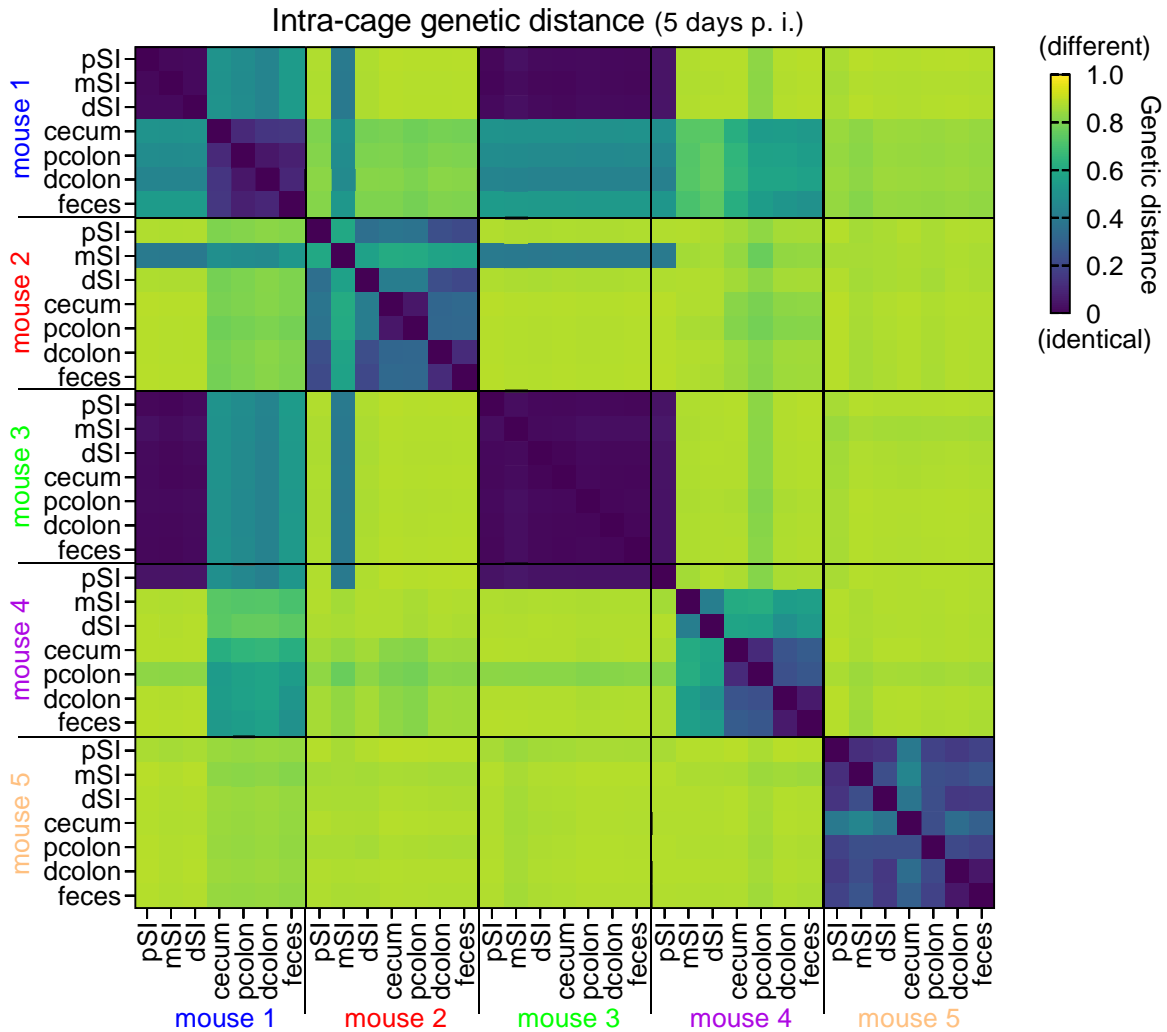


Supplemental figure 2. Additional data related to shedding of C57BL/6J mice in Figure 2.

In C57BL/6J mice inoculated with a high enough dose to cause infection ($\geq 10^8$ CFU), dose impacts the lag between inoculation and peak shedding (**a**) and the lag between inoculation and clearance (**d**) but does not change the days of peak shedding (**b**) or the total fecal burden (**c**). 4 animals per dose. (**a**, **b**, **d**) Arithmetic mean, (**c**) geometric mean.

Source data are provided as a Source Data file.

Supplement 3



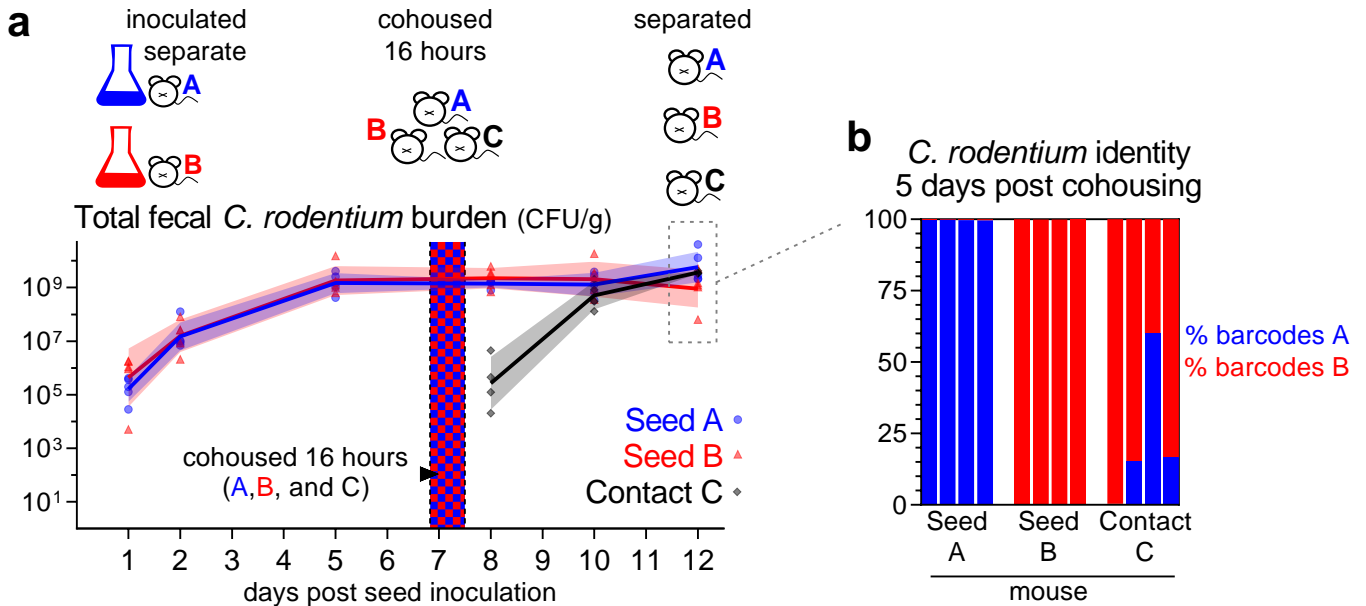
Supplemental figure 3. All intra-cage relationships from Figure 4a-e.

Comparison by genetic distance of *C. rodentium* populations 5 days post inoculation in 5 cohoused (intra-cage) C57BL/6J mice inoculated with STAMP-CR253. Zero indicates no difference between populations (identical).

From proximal (p) to distal (d): p small intestine (SI), mid (m) SI, dSI, cecum, pcolon, dcolon, feces.

Source data are provided as a Source Data file.

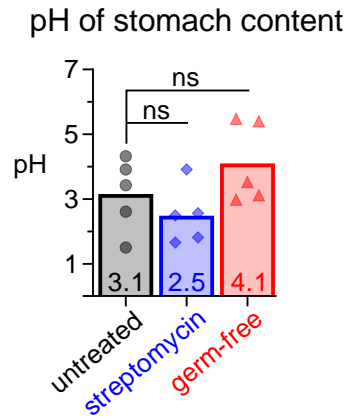
Supplement 4



Supplemental figure 4. Super-colonization resistance prevents cohoused mice from sharing *C. rodentium* populations.

To test transmission of *C. rodentium* to already infected mice, two cages of 4 C57BL/6J mice (seeds A and B) were separately inoculated with mixed pools of barcoded strains (barcodes A and B). Barcodes A and B are each a group of 3 separate strains with non-redundant barcodes. Seed animals A and B were separated until peak colonization. On the 6th day after seed inoculation, the mice were re-arranged to cohause 1 mouse each of seed A, seed B, and uninfected contact C overnight for 16 hours. The next morning, they were separated, housing the A seeds together, the B seeds together, and the C contacts together. 12 days post seed inoculation (5 days post cohousing) the fecal *C. rodentium* populations were analyzed to determine the contribution from pools A and B. <1% of reads from seed A mice were contributed by the B barcodes and vice versa. (a) Shedding data is presented as geometric means and standard deviations. Source data are provided as a Source Data file.

Supplement 5



Supplemental figure 5. The effect of microbiota on gastric acid.

Conventional, untreated C57BL/6J mice have similar stomach acidity as mice treated for 3 days with the antibiotic streptomycin (as in Figure 6) or mice lacking a microbiota (germ-free, as Figure 7). pH measured post-mortem in aspirated stomach fluid. Boxes are arithmetic means. One-way ANOVA adjusted p -values 0.54 (streptomycin) and 0.32 (germ-free), not significant (ns). 5 animals per group. Source data are provided as a Source Data file.

Supplemental table 1

microbiota	days p. i.	cage	mouse	variation	genomic change	annotation	coding change	amino acid change	
SPF C57BL/6J	5 days	A	A1	complex	g.3081146..3081467	intergenic			
			A2	none					
			A3	SNV	g.388509C>G	<i>lamB</i>	c.766G>C	p.Val256Leu	
			A4	none					
		B	B1	none					
			B2	none					
			C	C1	SNV	g.527543C>T	<i>panE</i>	c.232G>A	p.Asp78Asn
				C2	none				
C3	SNV	g.330872T>C		<i>ROD_03001</i>	c.557A>G	p.Glu186Gly			
	SNV	g.4274290A>G		<i>dgoT</i>	c.442A>G	p.Arg148Gly			
C4	none								
germ-free C57BL/6J	20 days	C	C1	deletion	g.3100349..3180553	multiple (including LEE)			
				SNV	g.4029101C>T	<i>glpK</i>	c.1000C>T	p.Pro334Ser	
				SNV	g.5295383C>T	intergenic			
			C2	deletion	g.3100349..3180553	multiple (including LEE)			
				SNV	g.4029101C>T	<i>glpK</i>	c.1000C>T	p.Pro334Ser	
				SNV	g.5295383C>T	intergenic			
			C3	deletion	g.3100349..3180553	multiple (including LEE)			
				SNV	g.4029101C>T	<i>glpK</i>	c.1000C>T	p.Pro334Ser	
				SNV	g.5295383C>T	intergenic			
		C4	deletion	g.3100349..3180553	multiple (including LEE)				
			SNV	g.4029101C>T	<i>glpK</i>	c.1000C>T	p.Pro334Ser		
			SNV	g.5295383C>T	intergenic				
		D	D1	SNV	g.4270455T>A	<i>dgoR</i>	c.143T>A	p.Val48Glu	
				SNV	g.4274290A>G	<i>dgoT</i>	c.442A>G	p.Arg148Gly	
				SNV	g.4634927C>T	<i>gntU</i>	c.895C>T	non-coding	
				insertion	g.5407322^5407357	intergenic			
		E	E1	deletion	g.3100354...319782 3	multiple (including LEE)			
				insertion	g.4989831^4989834	<i>gatZ</i>			
		F	F1	deletion	g.3100357..3198048	multiple (including LEE)			
				SNV	g.3937003C>T	<i>purH</i>	c.837C>T	non-coding	
				SNV	g.4274290A>G	<i>dgoT</i>	c.442A>G	p.Arg148Gly	
		G	G1	deletion	g.2391346..2391356	<i>galS</i>			
				complex	g.3174563..3174662	<i>grlR</i>			

Supplemental table 1. Changes in the *C. rodentium* genome following mouse expansion.

Whole genome sequencing was used to detect variations in the *C. rodentium* genome after expansion and selection in mice. 3 clones (colonies) from the STAMP-CR69K library were compared to isolates from the feces of infected mice (1 colony per mouse). No variants (“none”) detected in 4/6 isolates from SPF (specific pathogen free) animals 5 days p. i., 2/4 isolates from germ-free animals 5 days p. i., and 0/8 isolates from germ-free animals 20 days p.i. 4 cohoused germ-free animals 20 days p. i. (C1-4) had identical (clonal) isolates. These data were filtered to only include high confidence variants (present in >90% of reads).

Supplemental table 2

Aim	5' -> 3'
Clone ~800bp of <i>C. rodentium</i> intergenic DNA into pKD4	cttcggaataggaactaaggaggatattcaGTCTGACGCCGGTATCTGTA ATGC
	acggctgacatgggaattagccatggtccaAAAAAGCGCCGGGTGGCG
Check insertion into pKD4	gcgggactctggggttcgaa
	cgcagttcaacctgttgata
Clone <i>C. rodentium</i> ~800bp and KanR from pKD4 into pDS132 with 20n	ATATGTGATGGGTAAAAAGGATCGATCCTnnnnnnnnnnnnnnnn nnnnntcacgctgccgcaagcactc
	cgggagagctcgatatcgcatgcggtacctaaaaagcgccgggtggcggcttcgc
Check insertion into pDS132	CAGAAAATATCATAATATCT
	ACATGTGGAATTGTGAGCGG
	GATTTGCAGACTACGGGCCTAAAG
	GTGAGCGGATAACAATTTGTGG
Check integration into genome	GATTTGCAGACTACGGGCCT
	ctggaggatgaggggcggtatgattggtcgaacctctgc

Supplemental table 2. Primers used in the creation of STAMP-CR253.

Supplemental table 3

Name	5' -> 3'
AD001	CAAGCAGAAGACGGCATAACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD002	CAAGCAGAAGACGGCATAACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD003	CAAGCAGAAGACGGCATAACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD004	CAAGCAGAAGACGGCATAACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD005	CAAGCAGAAGACGGCATAACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD006	CAAGCAGAAGACGGCATAACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD007	CAAGCAGAAGACGGCATAACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD008	CAAGCAGAAGACGGCATAACGAGATCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD009	CAAGCAGAAGACGGCATAACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD010	CAAGCAGAAGACGGCATAACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD011	CAAGCAGAAGACGGCATAACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGAT CCTTGGCGGCAAGAAA
AD012	CAAGCAGAAGACGGCATAACGAGATTACAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD013	CAAGCAGAAGACGGCATAACGAGATTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD014	CAAGCAGAAGACGGCATAACGAGATGGAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGAT CCTTGGCGGCAAGAAA
AD015	CAAGCAGAAGACGGCATAACGAGATTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD016	CAAGCAGAAGACGGCATAACGAGATGGACGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGAT CCTTGGCGGCAAGAAA
AD018	CAAGCAGAAGACGGCATAACGAGATGCGGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGAT CCTTGGCGGCAAGAAA
AD019	CAAGCAGAAGACGGCATAACGAGATTTTACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGATC CTTGGCGGCAAGAAA
AD020	CAAGCAGAAGACGGCATAACGAGATGGCCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGAT CCTTGGCGGCAAGAAA
Var11	AATGATACGGCGACCACCGAGATCTAAGTCTTTCCCTACACGACGCTCTTCCGATCTGATGGGTAA AAAGGATCGATCC
Var12	AATGATACGGCGACCACCGAGATCTAAGTCTTTCCCTACACGACGCTCTTCCGATCTAGATGGGTAA AAAAGGATCGATCC
Var13	AATGATACGGCGACCACCGAGATCTAAGTCTTTCCCTACACGACGCTCTTCCGATCTTACGATGGGT TAAAAGGATCGATCC
Var14	AATGATACGGCGACCACCGAGATCTAAGTCTTTCCCTACACGACGCTCTTCCGATCTGCTAGATGGG TAAAAGGATCGATCC
Var15	AATGATACGGCGACCACCGAGATCTAAGTCTTTCCCTACACGACGCTCTTCCGATCTCGACTGGATG GGTAAAAGGATCGATCC
Var16	AATGATACGGCGACCACCGAGATCTAAGTCTTTCCCTACACGACGCTCTTCCGATCTTAGCGTAGAT GGGTAAAAGGATCGATCC
Var17	AATGATACGGCGACCACCGAGATCTAAGTCTTTCCCTACACGACGCTCTTCCGATCTTGCTGAGTCG ATGGGTAAAAGGATCGATCC
Var18	AATGATACGGCGACCACCGAGATCTAAGTCTTTCCCTACACGACGCTCTTCCGATCTGCTGATAGTA GATGGGTAAAAGGATCGATCC
Var19	AATGATACGGCGACCACCGAGATCTAAGTCTTTCCCTACACGACGCTCTTCCGATCTCAACTGCGTA GCGATGGGTAAAAGGATCGATCC

Var20	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATGACCGTCT CGAGATGGGTAAAAAGGATCGATCC
var21	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAATGATGGGT TAAAAAGGATCGATCC
var22	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATGCGATGGG TAAAAAGGATCGATCC
var23	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCACGATGG GTAAAAAGGATCGATCC
var24	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCATTGATG GGTAAAAAGGATCGATCC
var25	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGAATCGAGAT GGGTAAAAAGGATCGATCC
var26	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCAACTTGAT GGGTAAAAAGGATCGATCC
var27	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGCGTGGCG ATGGGTAAAAAGGATCGATCC
var28	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTGTACCTT GATGGGTAAAAAGGATCGATCC

Supplemental table 3. Primers used for STAMP sequencing.