Journal Club

April 10th 2015

1st Paper :

Manipulation of the Quorum Sensing Signal AI-2 Affects the Antibiotic-Treated Gut Microbiota

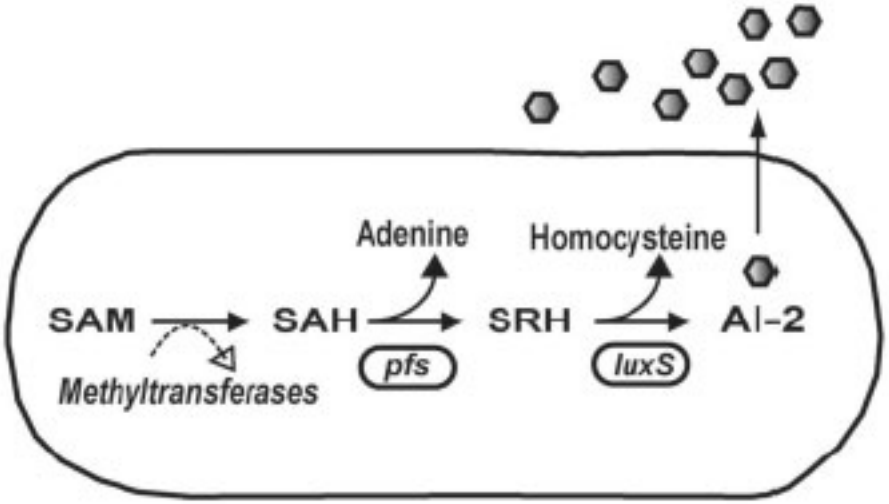
Jessica Ann Thompson, Rita Almeida Oliveira, Ana Djukovic, Carles Ubeda, Karina Bivar Xavier

Cell Reports (March 2015)

Quorum sensing – Cell-to-cell communication in bacteria

Quorum sensing (QS) is a widespread process in bacteria that employs autoinducing chemical signals to coordinate diverse, often cooperative activities.

AI-2 production



AI-2 sequestration and regulation

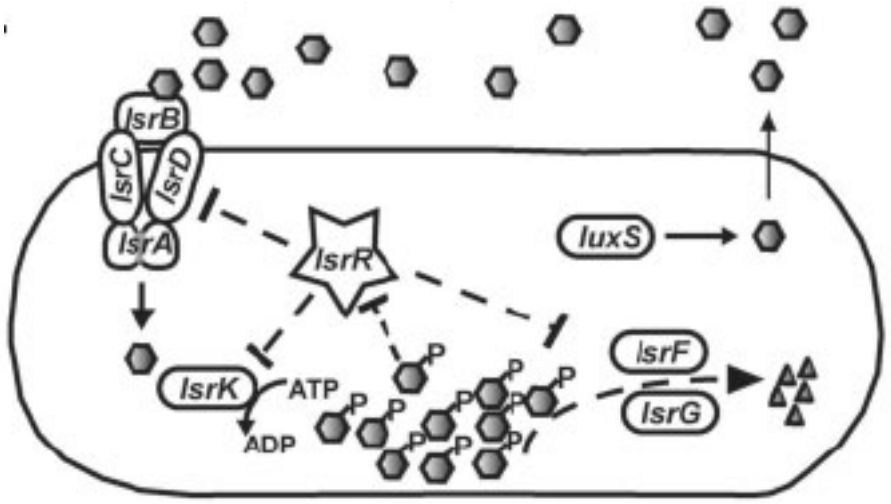


Figure 1: *E. coli* Accumulate and Deplete AI-2 in the Gut of Mono-colonized Mice

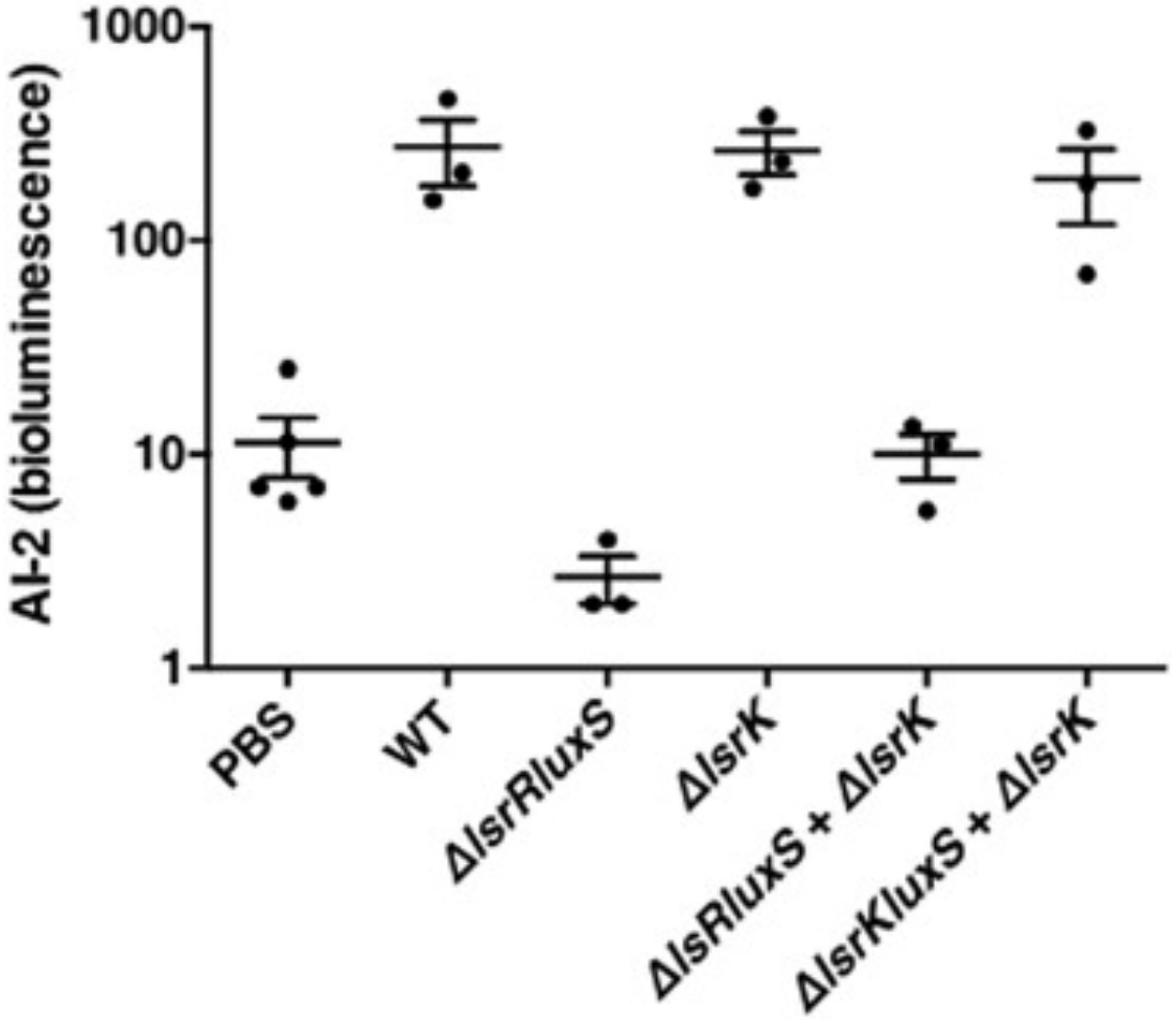
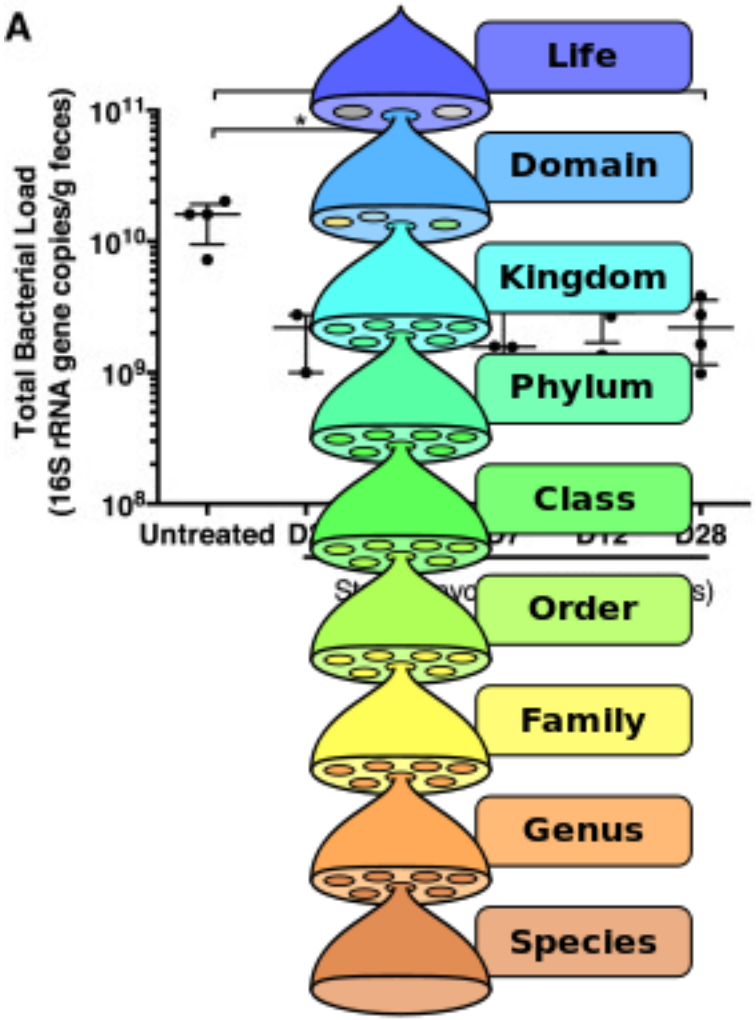
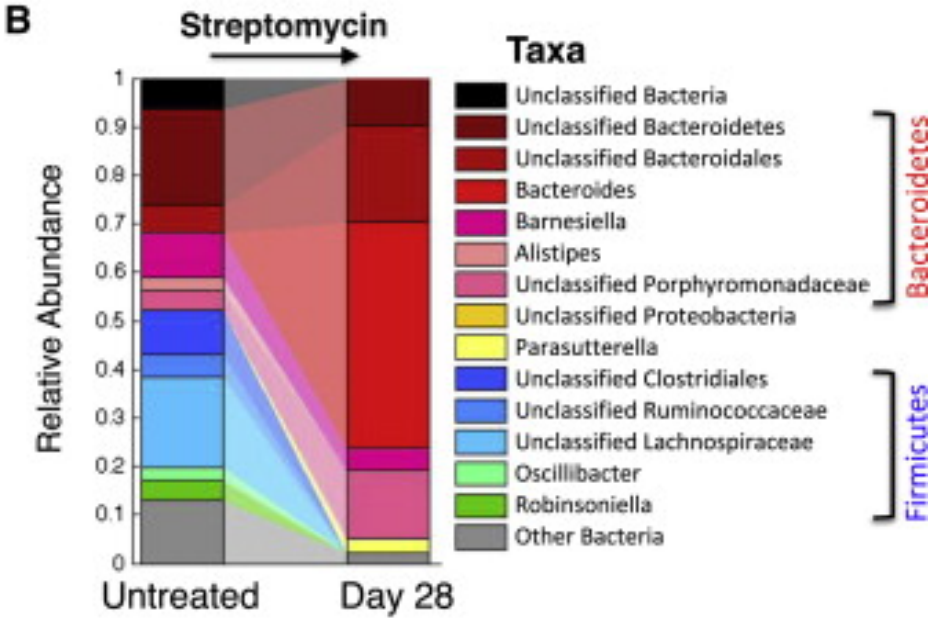


Figure 2: Streptomycin Changes Intestinal Microbiota Load and Composition





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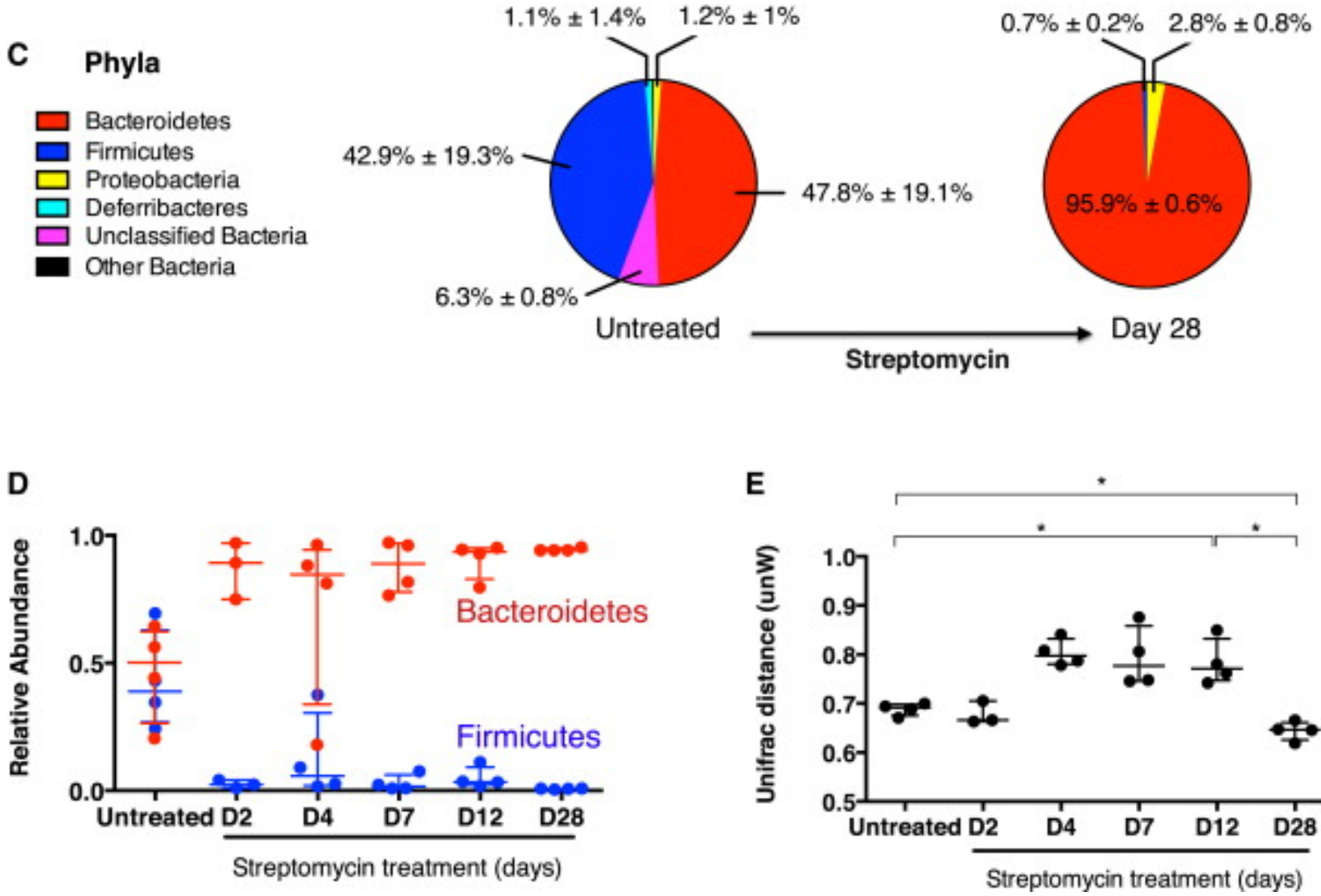


Figure 3 : *E. coli* Colonization Levels and Total Microbiota Load in Streptomycin-Treated Mice

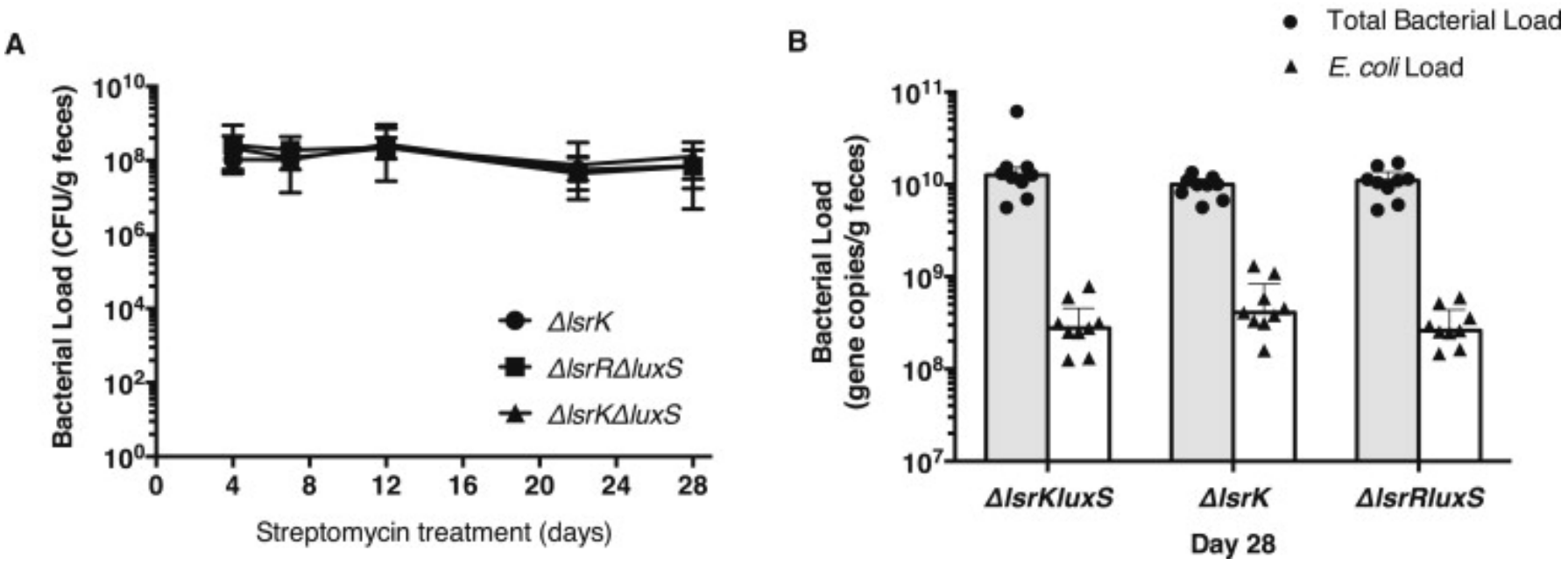
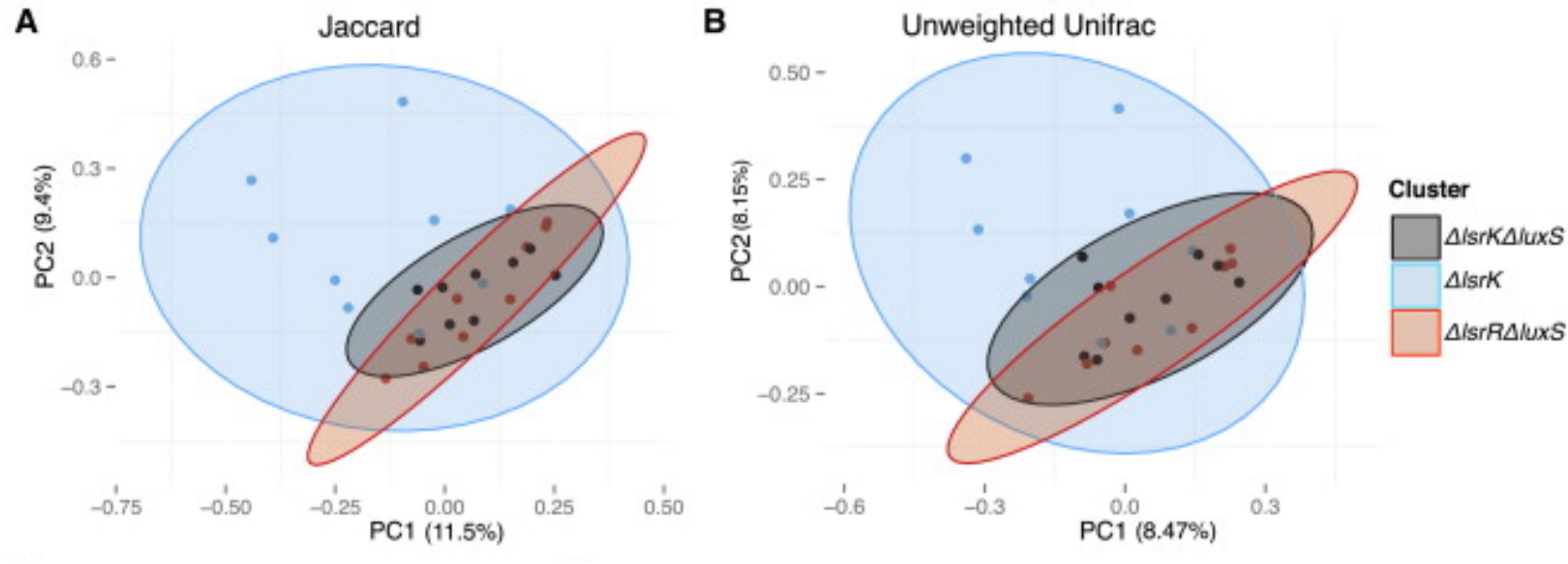


Figure 4 : Microbiota Composition of Streptomycin-Treated Mice Differs in the Presence of *ΔlsrK* Mutant *E. coli*



Ellipses centered on the categorical averages of the metric distances with a 95% confidence interval for the first two coordinates of each group were drawn on the associated PCoA

Figure 4 : Microbiota Composition of Streptomycin-Treated Mice Differs in the Presence of *ΔlsrK* Mutant *E. coli*

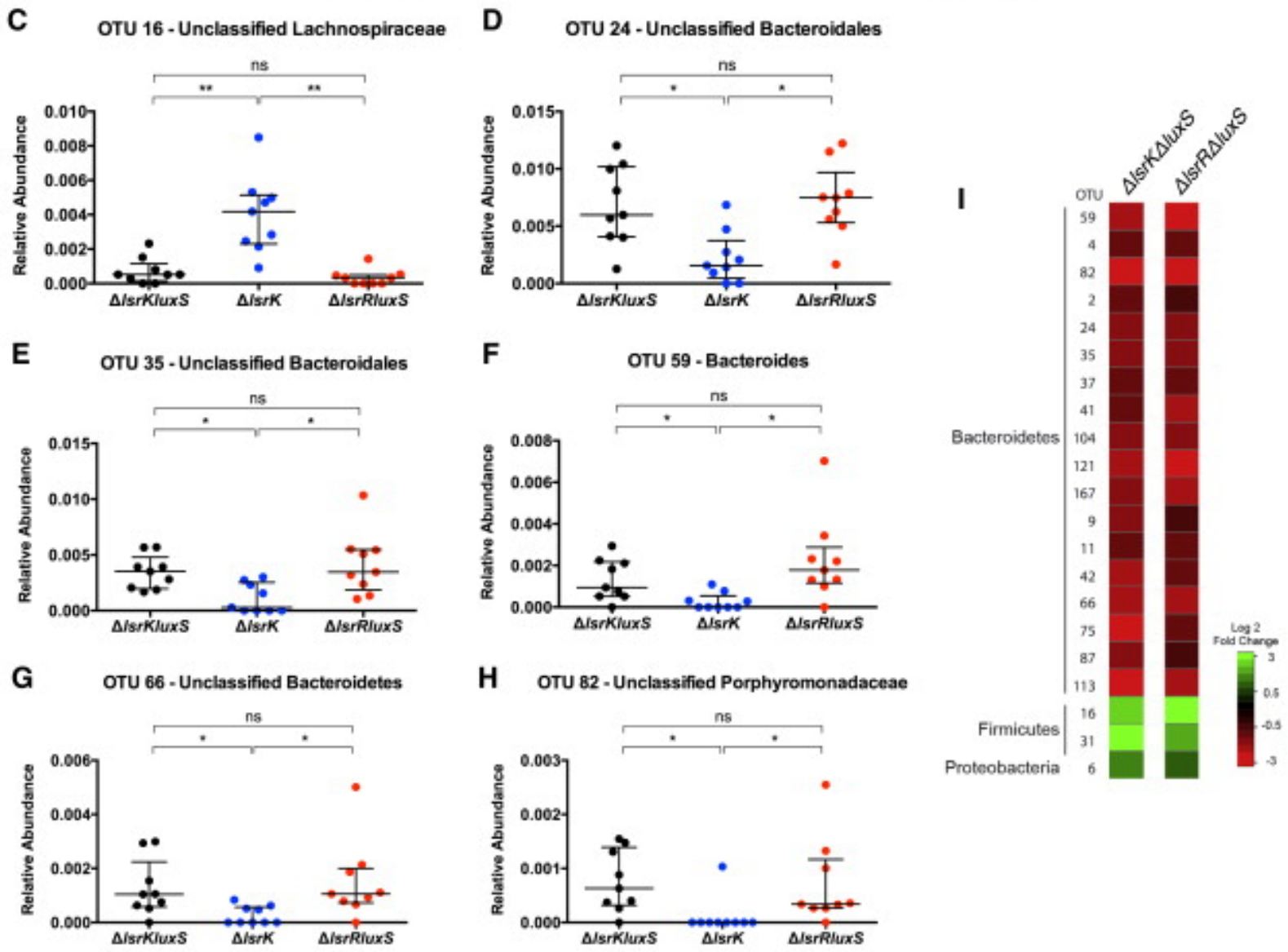


Figure 5: Colonization with *ΔlsrK* Mutant Bacteria Changes the Relative Abundance of the Major Phyla

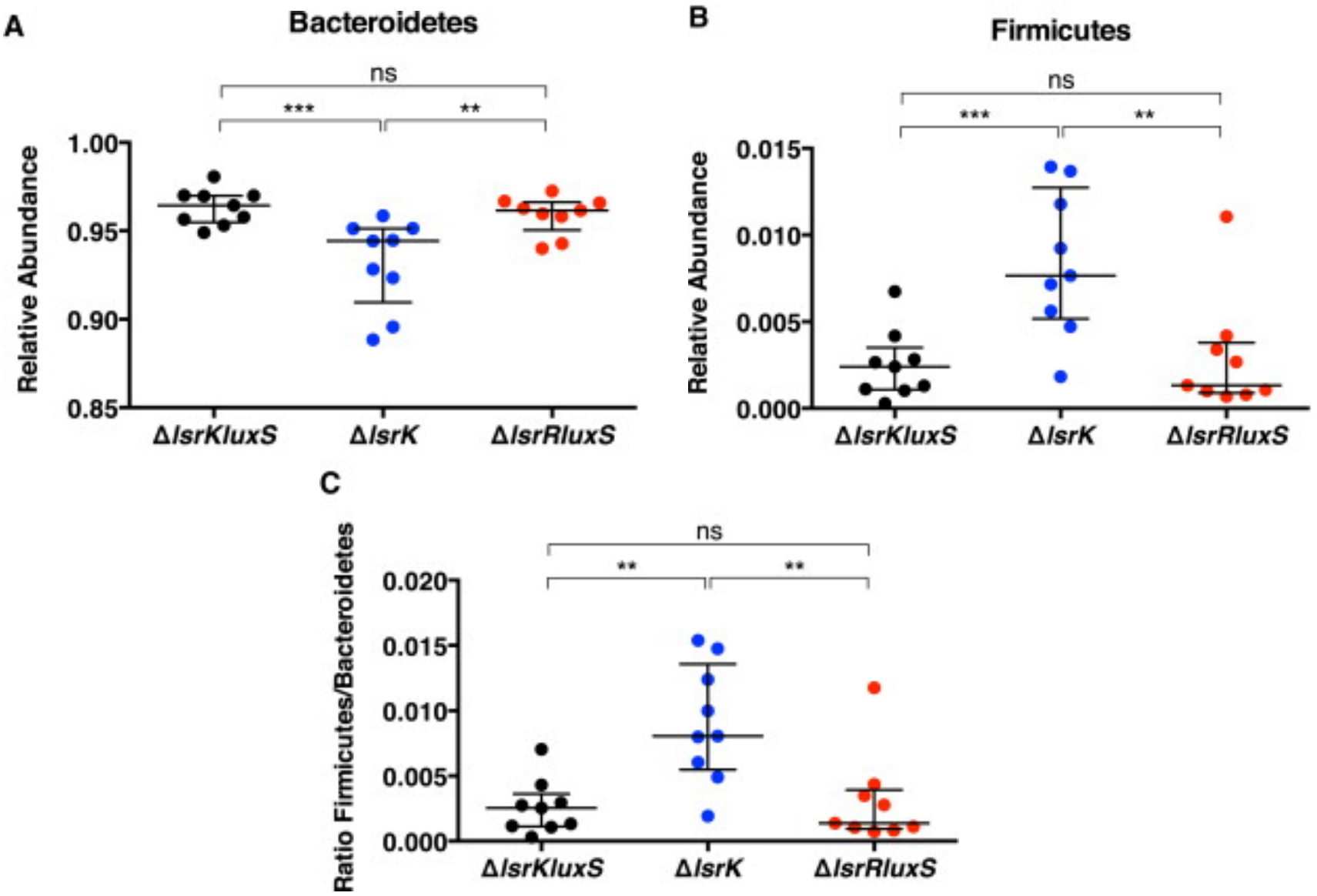
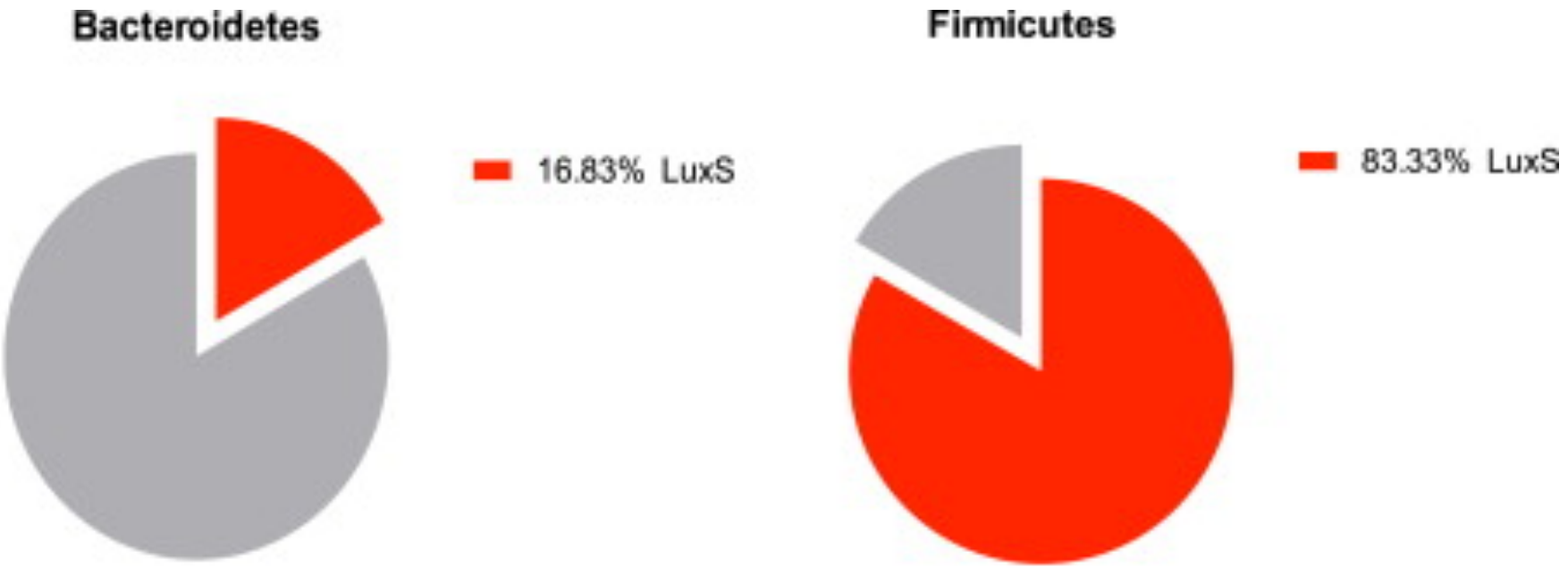
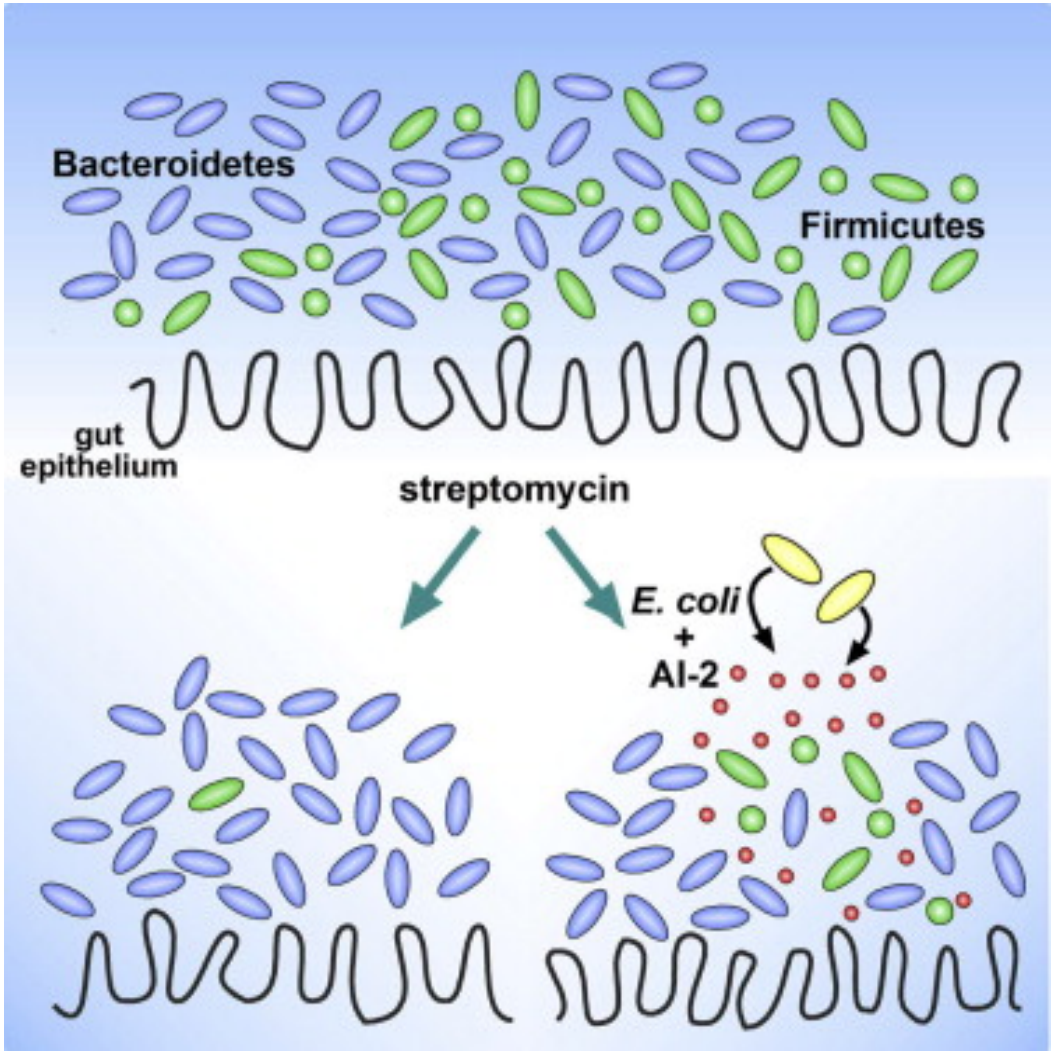


Figure 6 : Higher Prevalence of *LuxS* Orthologs in the Complete Genomes of Bacteria Belonging to the Firmicutes



	Putative LuxS gene (%)
Bacteroidetes	16.83
Firmicutes	83.33
- Bacilli	96.80
- Clostridia	48.68
- Other	27.27

Summary


Bacteroidetes
Firmicutes
gut
epithelium
streptomycin
E. coli
+
AI-2

2nd Paper :

Improving microbial fitness in the mammalian gut by *in vivo* temporal functional metagenomics

Stephanie J Yaung[1,2,3], Luxue Deng[4], Ning Li[4], Jonathan L Braff[3], George M Church[2,3], Lynn Bry[4], Harris H Wang[5,6,†,*] & Georg K Gerber[4,†,**]

Molecular Systems Biology (2015)

Bridging the knowledge gap: from microbiome composition to function

Jeremiah J Faith

Molecular Systems Biology (2015)

Advantages / Issues with gain-of-function *in vivo* screening method

- Can study essential genes
- Can study synergistic effects with the host
- Using E. coli as a host species for the library allows the study of genes from intractable and even un-cultivable organisms

- Still requires reasonable DNA input
- Functions studied are dependent on the host gene network (operons incomplete etc…)

Figure 1 : Experimental design

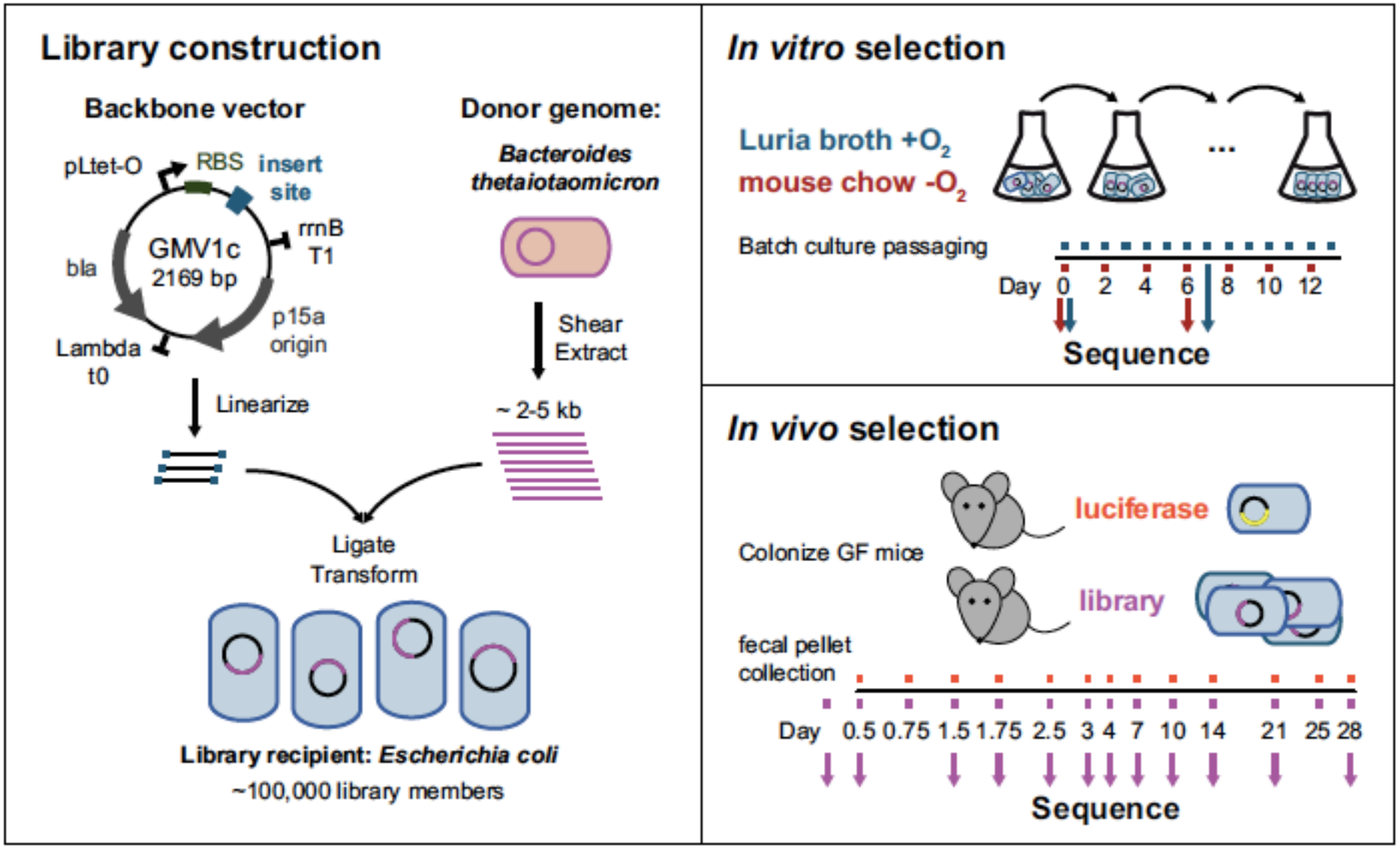


Figure 2 : Input library characterization

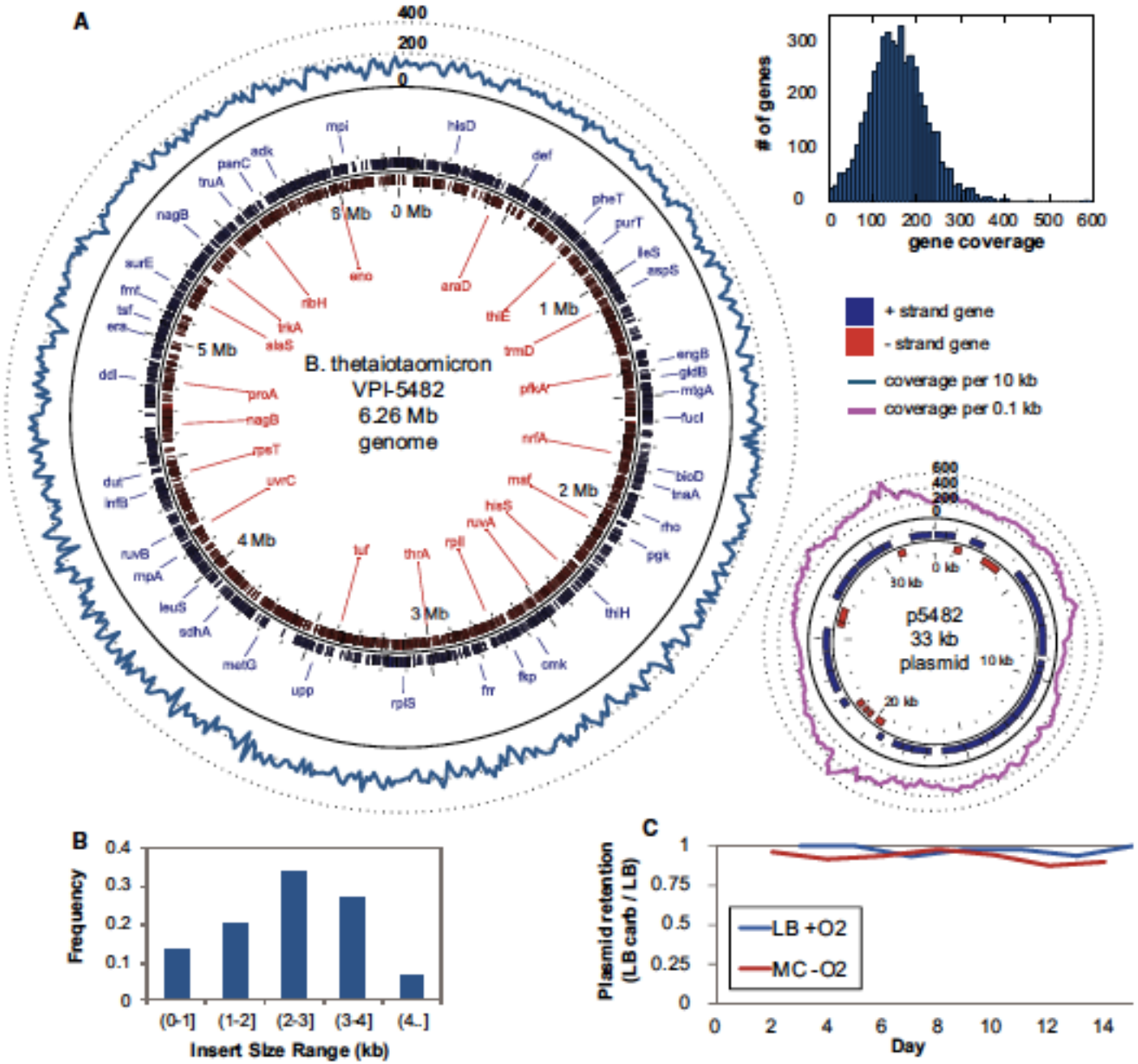


Figure 3 : *In vivo* selection experiments

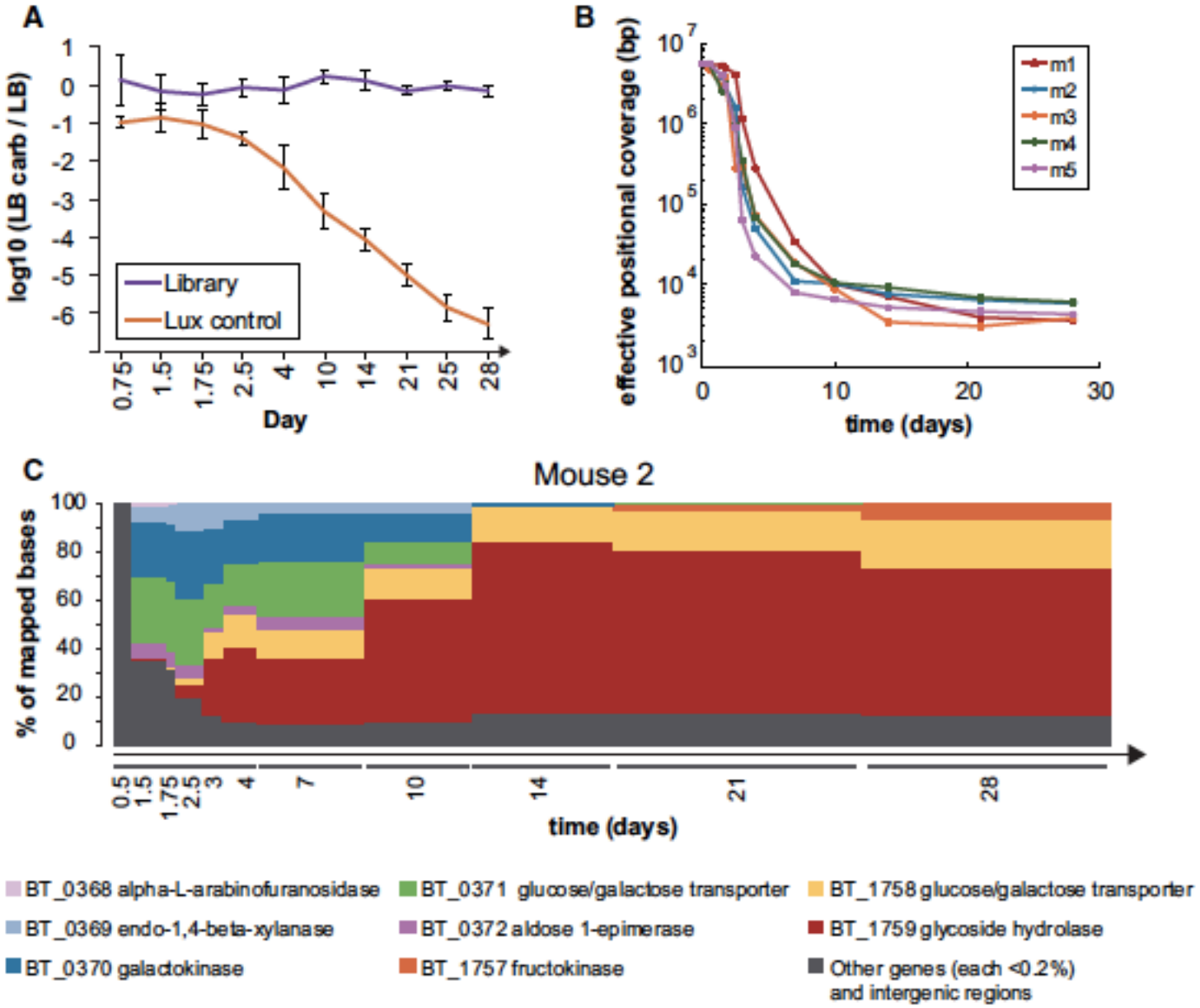
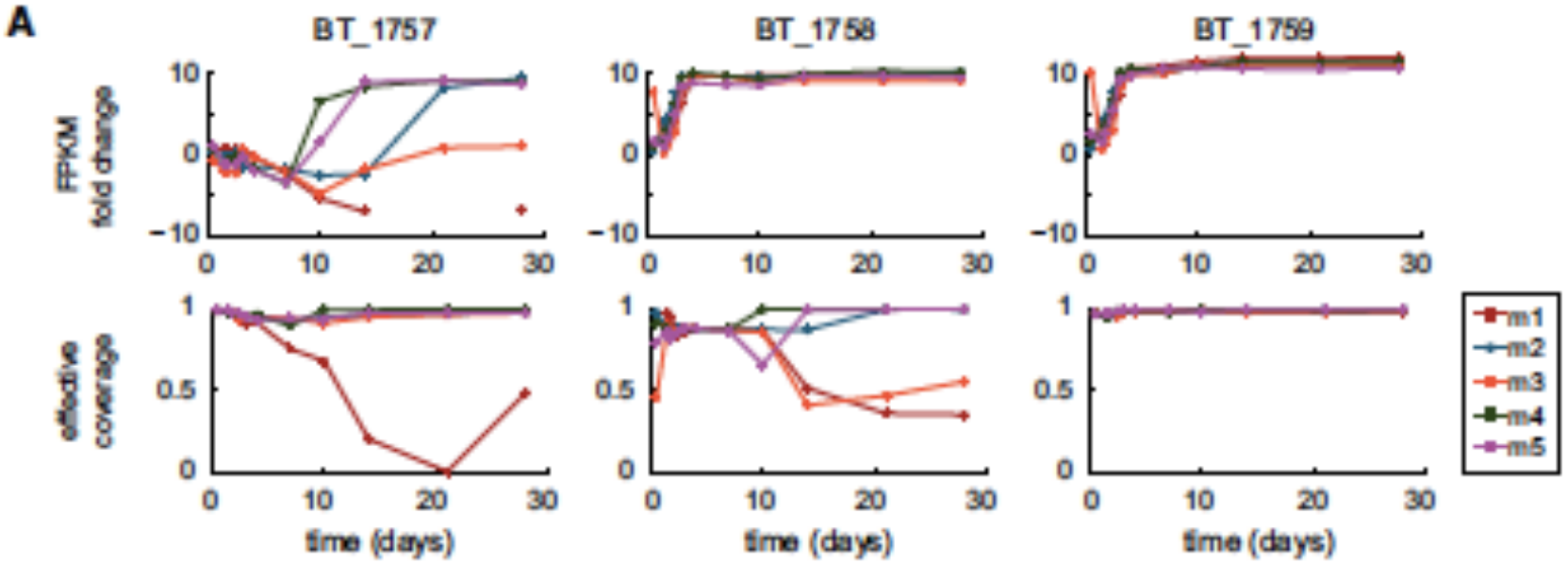


Table 1. Statistical testing of *in vivo* selection of Bt genes.

Gene	Annotation	TA-RA *q*-value	TA-NEC *q*-value
BT_0297	outer membrane lipoprotein SilC	3.06E-02	4.08E-04
BT_0370	galactokinase	1.14E-03 (3.25E-03)	5.94E-06 (6.95E-09)
BT_0371	glucose/galactose transporter	1.14E-03 (3.14E-03)	3.50E-02 (4.21E-05)
BT_0477	D-glycero-alpha-D-manno-heptose-1,7-bisphosphate 7-phosphatase (*gmhB*)	1.67E-02	1.32E-02
BT_0478	hypothetical protein	1.77E-03	2.47E-02
BT_1510	hypothetical protein	3.86E-02	1.10E-03
BT_1511	outermembrane protein OmpA	4.38E-02	7.33E-04
BT_1730	dTDP-4-dehydrorhamnose reductase (*rfbD*; *rmlD*)	3.86E-02	3.45E-04
BT_1731	hypothetical protein	4.38E-02	8.80E-03
BT_1757	fructokinase	1.58E-02	2.50E-03
BT_1759	glycoside hydrolase	1.19E-02 (2.48E-07)	2.58E-04 (1.21E-09)
BT_1771	cell surface protein	4.38E-02	4.00E-03
BT_4265	GMP synthase (*guaA*)	3.86E-02	3.33E-02

Figure 4 : BT_3759 glycoside hydrolase



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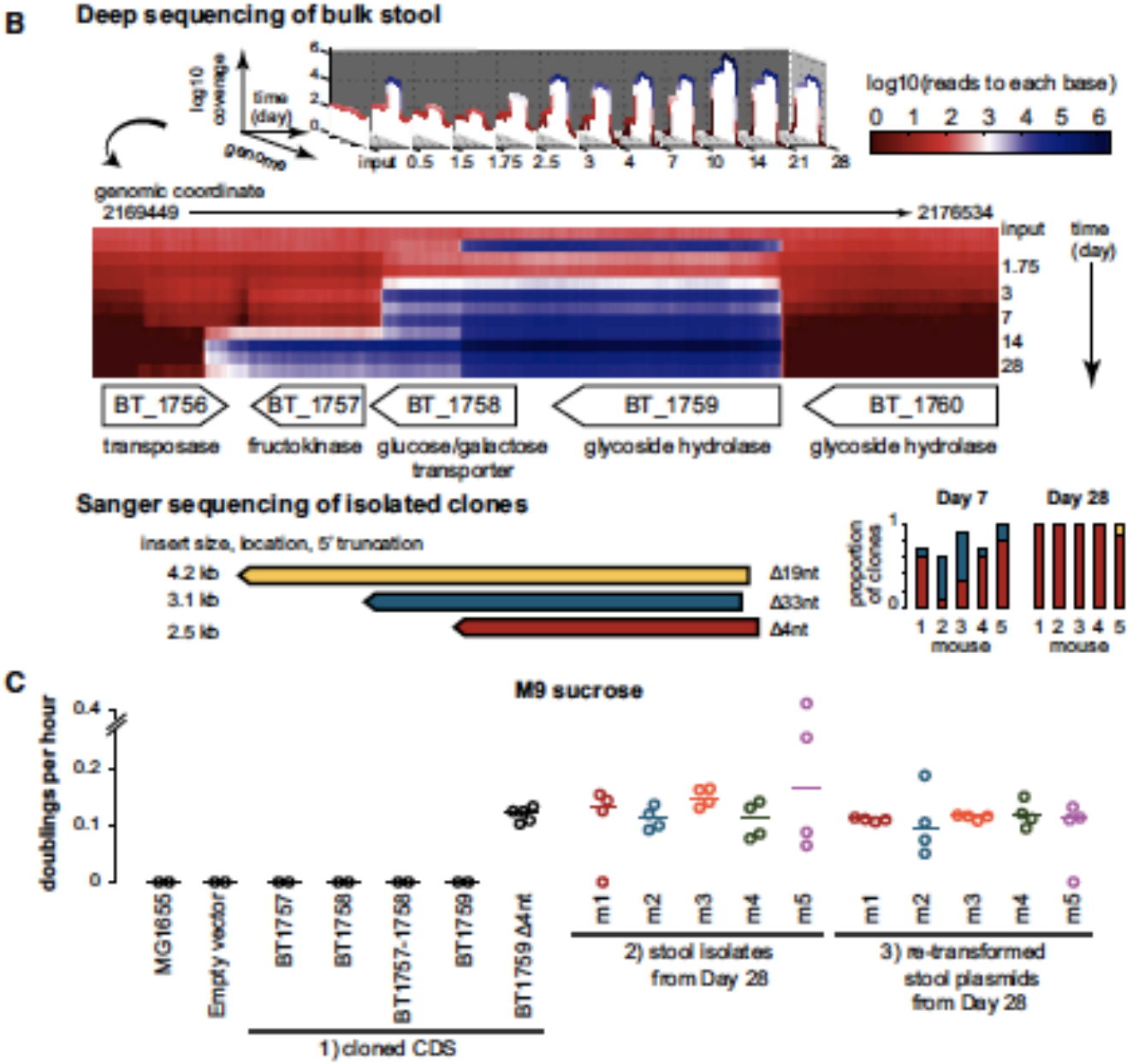


Figure 5 : BT_0370 galactokinase and BT_0371 glucose/galactose transporter

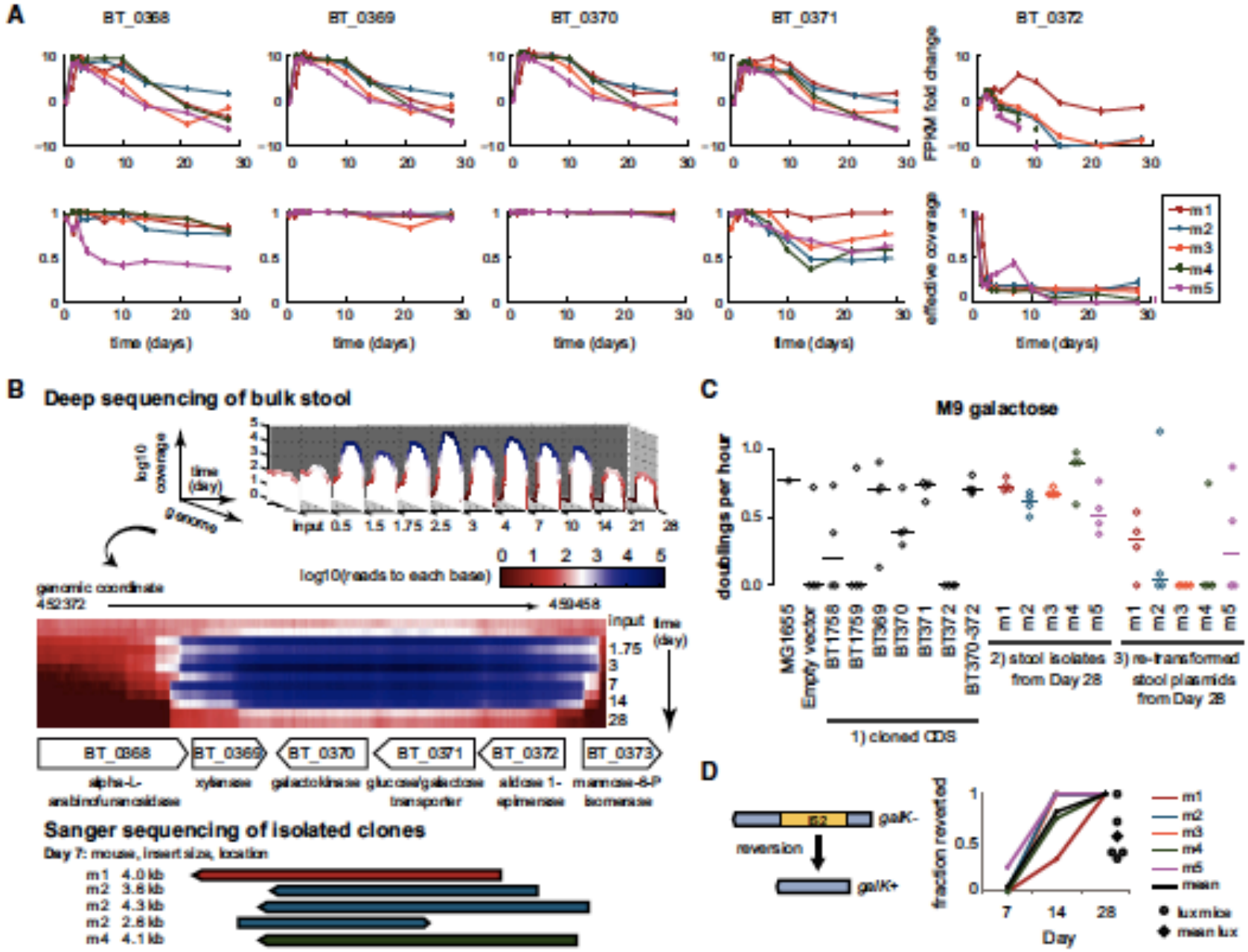
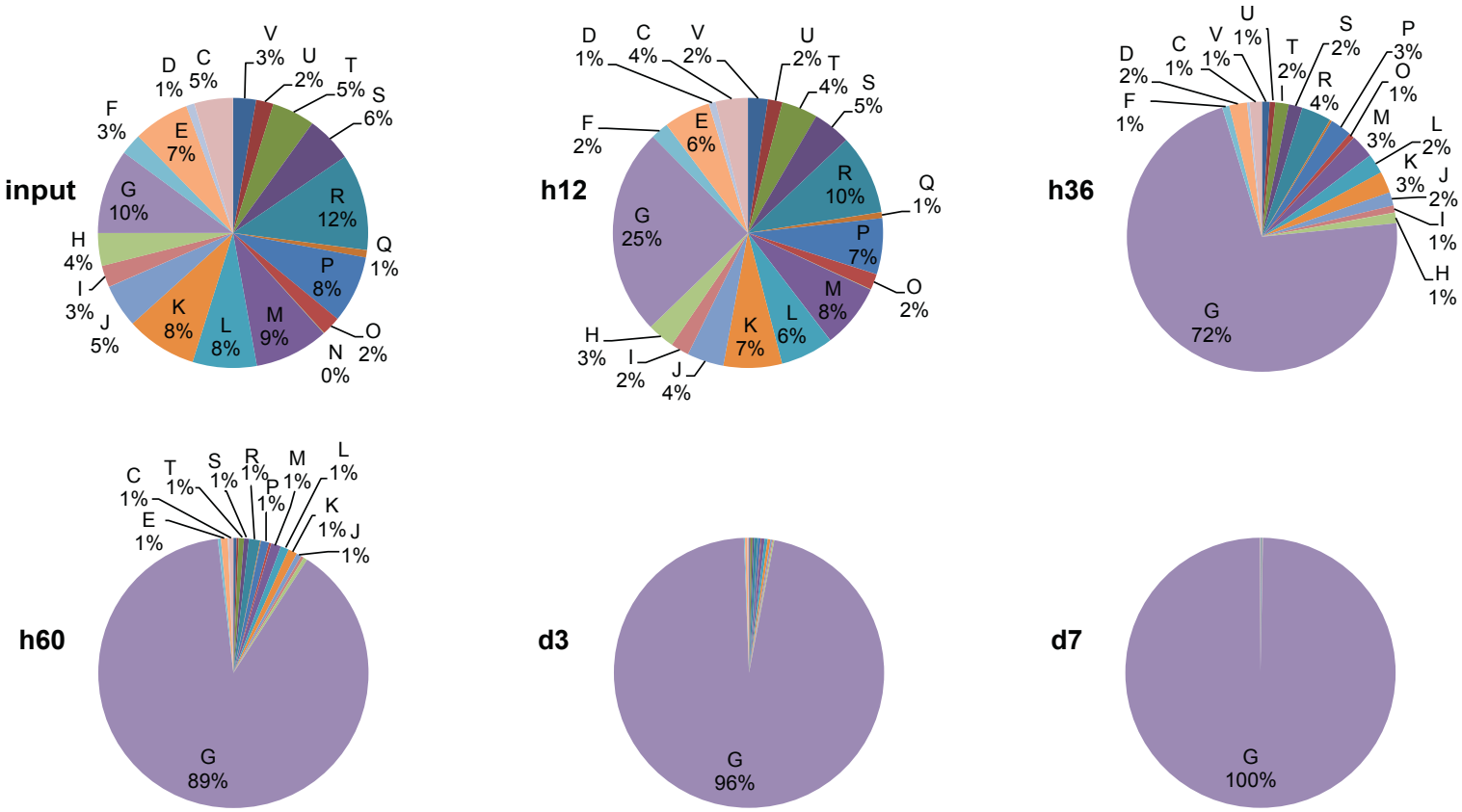


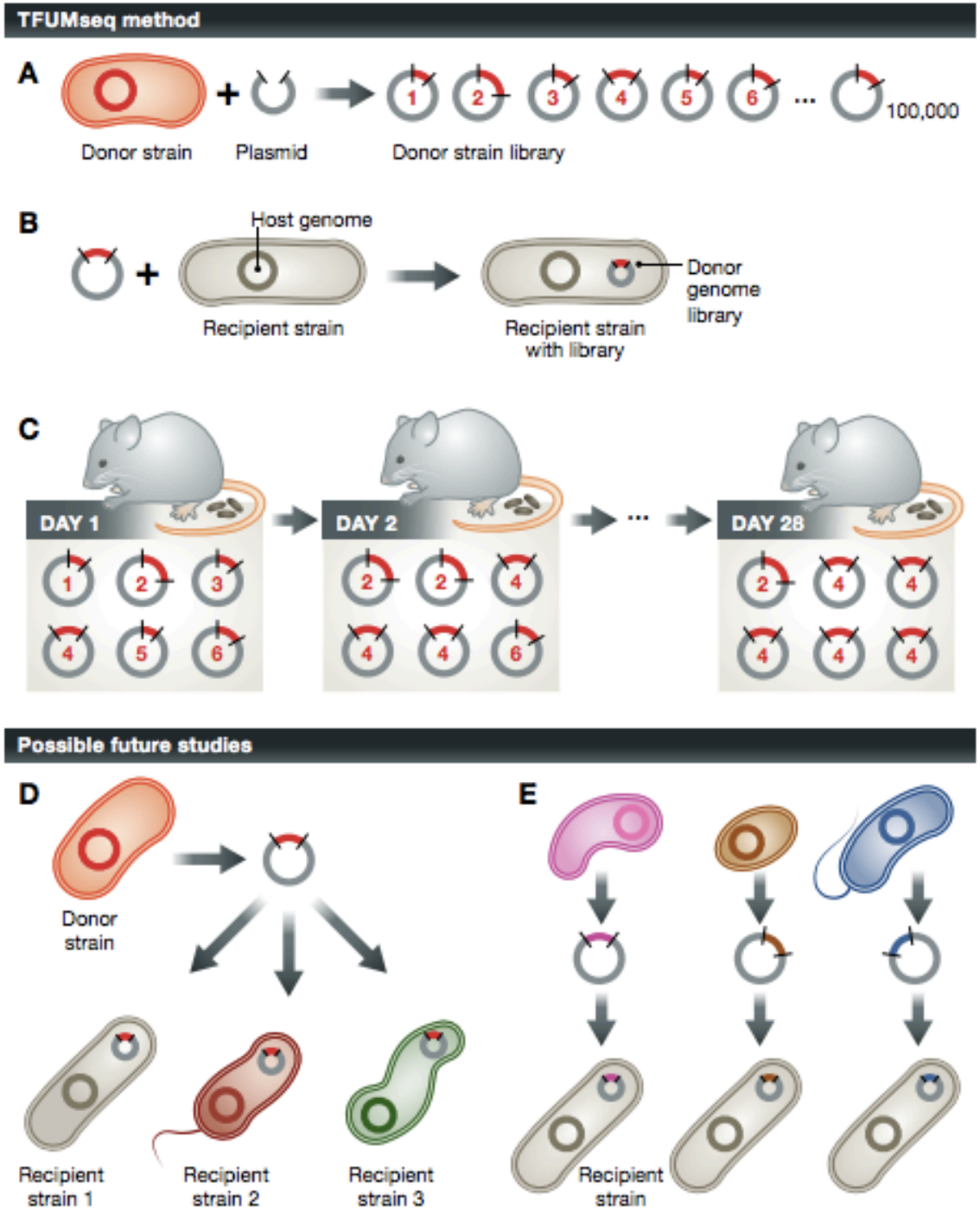
Table 2. Genetic variants in mouse-isolated clones identified by whole-genome sequencing.

Sample	Insert locus and size (kb)	Genomic galK+/–	Variant position on *Escherichia coli* genome	Variant impact and coverage
NEB Turbo control	–	galK–		
Day 7 Mouse 1 clone 1	BT_1759 (2.5)	galK–		
Day 7 Mouse 1 clone 3	BT_0370 (4.0)	galK–	SNV 2976657 G>T	galR (R20L) [34/34 reads]
Day 7 Mouse 2 clone 5	BT_0370 (4.3)	galK–		
Day 7 Mouse 3 clone 1	BT_1759 (3.1)	galK–		
Day 7 Mouse 4 clone 4	BT_0370 (4.1)	galK–		
Day 7 Mouse 5 clone 2	BT_1759 (2.5)	galK+		
Day 7 Mouse 5 clone 4	BT_1759 (3.1)	galK–		
Day 28 Mouse 1 clone 1	BT_1759 (2.5)	galK+	SNV 363100 A>G	lacY (F27S) [173/173 reads]
Day 28 Mouse 2 clone 1	BT_1759 (2.5)	galK+		
Day 28 Mouse 3 clone 1	BT_1759 (2.5)	galK+		
Day 28 Mouse 4 clone 1	BT_1759 (2.5)	galK+		
Day 28 Mouse 5 clone 1	BT_1759 (2.5)	galK+		
Day 28 Mouse 5 clone 4	BT_1759 (4.2)	galK+		
Day 28 Mouse 7 clone 1 lux control	–	galK+		
Day 28 Mouse 10 clone 2 lux control	–	galK–	1 SNV 3993675G>A 2 SNV 780994G>A 3 F plasmid SNV 67772 T>C	1 cyaA (G175S) [122/122 reads] 2 intergenic, between lysW and valZ [108/108 reads] 3 traY promoter (–35) [229/229 reads]

Supp Figure 4 : COG distribution of sequenced library



- [C] Energy production and conversion
- [D] Cell cycle control, cell division, chromosome partitioning
- [E] Amino acid transport and metabolism
- [F] Nucleotide transport and metabolism
- [G] Carbohydrate transport and metabolism
- [H] Coenzyme transport and metabolism
- [I] Lipid transport and metabolism
- [J] Translation, ribosomal structure and biogenesis
- [K] Transcription
- [L] Replication, recombination and repair
- [M] Cell wall/membrane/envelope biogenesis
- [N] Cell motility
- [O] Posttranslational modification, protein turnover, chaperones
- [P] Inorganic ion transport and metabolism
- [Q] Secondary metabolites biosynthesis, transport and catabolism
- [R] General function prediction only
- [S] Function unknown
- [T] Signal transduction mechanisms
- [U] Intracellular trafficking, secretion, and vesicular transport
- [V] Defense mechanisms


TFUMseq method
A
Donor strain
Plasmid
Donor strain library
100,000
B
Host genome
Recipient strain
Donor genome library
Recipient strain with library
C
DAY 1
DAY 2
DAY 28
Possible future studies
D
Donor strain
Recipient strain 1
Recipient strain 2
Recipient strain 3
E
Recipient strain